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## SULFUR OXIDATION IN SOILS AND ITS EFFECT ON THE AVAILABILITY OF MINERAL PHOSPHATES<sup>1</sup>

By

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### INTRODUCTION

Early in the nineteenth century de Saussure (46) expressed the belief that the ash constituents of plants are indispensable for their growth. Among the ash constituents he detected the constant presence of phosphates and was, therefore, led to assume that phosphorus is an essential ingredient of plant-food. The thought that phosphorus plays a leading role in plant nutrition was further emphasized and developed by Liebig (31). He recognized that a deficiency of this element would necessarily limit production, as is clearly evident from his statement of the law of the minimum. "By the deficiency or absence," he said, "of one necessary constituent, all the others being present, the soil is rendered barren for all those crops to the life of which that one constituent is indispensable."

It was also Liebig's belief that plants could secure their nitrogen from the atmosphere in the form of ammonia, and that a sufficient supply of alkalis, sulfates and phosphates was, therefore, adequate for maximum production under favorable environmental conditions. Moreover, he was led to conclude from the examination of a number of analyses of plant ashes that the mineral requirements differed greatly with the character of the plants themselves and that crops should be supplied liberally with certain mineral ingredients in accordance with their requirements. In its main points Liebig's claim concerning the essential character of phosphorus and of other mineral ingredients in plant nutrition was confirmed by the experiments carried on at Rothamsted under the direction of Lawes and Gilbert. As stated by Russell (44) these experiments had, among other matters, demonstrated by 1855 that "crops require phosphates and salts of alkalis, but the composition of the ash does not afford reliable information as to the amounts of each constituent needed, *e. g.* turnips require

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large amounts of phosphates, although only little is present in their ash. The facts demonstrated by the investigations at Rothamsted are in accord with important evidence contributed from other sources. This evidence includes the numerous sand and water culture experiments performed among others, by Knop, Sachs, Stohmann, Nobbe, Rautenberg, Kühn, Lucanus, W. Wolff, Hampe, Beyer, E. Wolff, Wagner, Bretschneider and Lehmann (1).

In an empirical way the farmers of the Old World recognized the value of certain phosphatic fertilizers long before the beginning of modern research on soils and crops. It is probable that the phosphorus in animal bones and in fish was utilized in crop production in very ancient times. It is also probable that bones were used in England at least occasionally as early as the latter half of the 17th century. The more systematic use of bones was begun about one hundred years later. We are told that: "Col. St. Leger of Wormworth, was the first to introduce bone manuring into England in 1775; but it was many years before their value was fully understood, and it was only in connection with the turnip culture, that they came into general use" (19).

As the use of bones became more common it was recognized that they were slow and uncertain in their action. For instance, it is pointed out by Falkner (15) that: "The smaller the pieces into which the bones are broken, or the nearer to which they are reduced to powder, the more immediate will be their power over the production of the present crop. On clays they are now seldom employed as they show no immediate effect, and require more than light land, but they cannot fail to be ultimately beneficial to the soil." In order to increase the availability of the phosphorus in bone, fermentation in contact with manure and other organic substances was resorted to. In 1829 Anderson of Dundee introduced machinery for grinding bone (15) and thus made their use more effective. A still further step in advance in the utilization of bones as a fertilizer was made when Liebig suggested in 1840 the treatment of bones with sulfuric acid. This was the beginning of the manufacture of dissolved phosphates, or super-phosphates. Soon after that Lawes patented a process for the preparation of super-phosphates out of coprolites and other mineral phosphates. Thus the foundation was laid of a great industry which, in supplying enormous quantities of readily available phosphorus to crops, has made a deep impression on the agriculture of Europe and North America.

Bones served, at first, as the chief source of the super-phosphates sold in England. Later the animal black or animal charcoal from sugar refineries was used as a source of phosphorus. Finally when the possibilities of the large phosphate deposits in the United States were realized, work was started on the manufacture of commercial fertilizers with the mineral phosphates as a source of supply.

Some interesting information on the early history of the phosphate industry in the United States is furnished by Moses (38). According to him Prof. Shepard and Col. Hatch suggested, in 1859, "the utilization of phosphatic marls in the manufacture of commercial fertilizers, and started a factory at or near Charleston, which was, however, soon abandoned. Remains of their compost heap were utilized by neighboring farmers with good effect long after the war." It appears further, that Dr. St. Julien Ravenel of Charleston engaged in the manufacture of super-phosphates out of European phosphates, soon after the close of the Civil War. When phosphate deposits were discovered in South Carolina in 1867, several companies were organized for their utilization, among them Pratt and Holmes (Charleston Mining Company) and Ravenel and Dukes (Wands Company).

The phosphate deposits of Florida were discovered in 1887 and those of Tennessee in 1894. More recently the United States Geological Survey has prospected and located enormous deposits of phosphate rock in Idaho, Wyoming and Utah. It is estimated that there are in sight 200 million tons in Florida, about 90 million tons in Tennessee and about 30 million tons in South Carolina, Kentucky and Arkansas. These amounts appear insignificant when compared with the five billions of tons of phosphate rock already located in the western states. There is no doubt that other deposits will be located in the Northwest and elsewhere in the United States. But aside from the deposits in North America there are extensive deposits of phosphate rock in Northern Africa, in the Dutch West Indies, on some of the islands in the Southern Pacific, in European Russia and elsewhere.

#### *The Source of Phosphorus in Farm Practices*

Where artificial manures are used in European agriculture, acid phosphate, or superphosphate, is supplemented by basic slag, bone meal and phosphatic guano. From the standpoint of quantity only, the basic slag is of large importance. In the United States, on the other hand, basic slag is quite unimportant as a commercial source of phosphorus. By far the greatest proportion of this plant-food constituent is derived here from mineral phosphates after the latter have been acidulated with sulfuric acid. In 1914 there were produced in the United States 1,760,290 tons of superphosphates and 1,116,739 tons of ammoniated superphosphates, aside from the 4,483,465 tons of complete fertilizers. The total fertilizer output of that year was 8,414,959 tons. Among the raw materials employed in the manufacture of these fertilizers were included 2,080,961 tons of phosphate rock, 148,191 tons of bone meal and 1,973,054 tons of sulfuric acid. Since in the manufacture of superphosphate one ton each of sulfuric acid and phosphate rock are used to produce two tons of superphosphate it is evident that there was manufactured in 1914 an equivalent of about 4,000,000 tons of acid phosphate.

Obviously the cost of acidulation and of the acid itself is one of great economic importance. Acid phosphate usually contains 14 to 15 per cent of phosphoric acid, while the phosphate rock from which it is made contains about 30 per cent phosphoric acid. Hence it is apparent that the so-called available phosphoric acid in acid phosphate is more costly than the inert phosphoric acid in untreated phosphate rock. Furthermore, the greater concentration of the latter would make it relatively cheaper to transport than the acid phosphate. Hence, economy would counsel the use of finely ground phosphate rock, "floats," rather than of acid phosphate, provided that the former could be made to yield rapidly enough an adequate supply of phosphorus to the crop which it is intended to benefit. A discussion of the relative cost and effectiveness of floats and of acidulated phosphate rock would be out of place in this paper. Facts bearing on this question have received consideration among others in the publications of Hall (20), Hopkins (24), Wheeler (56) and Priianishnikov (43). At this time it may be pointed out merely that, while with some soils and crops floats may serve as the most economical and effective source of phosphorus, their use may be desirable only under a fairly limited range of conditions. The limitation of floats may be brought out more clearly in the following statements which seem to be warranted:

1. No natural phosphate is of much value applied directly to the soil except when it is in a state of very fine division.
2. Natural phosphates which consist chiefly of the calcium salt are of very little value when applied directly to soil deficient in organic matter or containing a very large amount of calcium carbonate.
3. Natural calcium phosphate in a finely ground state can be applied with great benefit to soil consisting essentially of vegetable mold or very rich in organic matter.
4. The phosphates of iron and aluminum are less useful than the natural phosphates of calcium.
5. With truck crops, and with most crops grown under intensive conditions raw rock phosphate is unsatisfactory as a phosphorus fertilizer.

In view of the cheapness of tricalcic phosphate as compared with acid phosphate, much thought has been given to the study of methods that could be economically used on the farm for increasing the availability of the former. Taking their cue from the earlier experience with bones, investigators in Europe and America have carried out a large number of composting experiments in which floats were kept for varying lengths of time in contact with decaying organic matter.

#### *Composting Experiments*

In 1873 Dietrich (14), who had noticed that applications of raw phosphates produced large increases in crop yield when used on moorland soils started some experiments for the purpose of determining whether the humic acids would have a solvent effect on the phosphates. He made

up a compost of peat and phosphate rock which was kept moist. Every 14 to 28 days the soluble phosphates were extracted with one liter of water. In a period of 10 to 11 months only 2 to 3 per cent of the phosphoric acid in the rock had become soluble.

A short time later Heiden and Hosaeus (22), working with a low grade phosphate, high in iron, containing 15.6 per cent of  $\text{Fe}_2\text{O}_3$  and 14.56 per cent of  $\text{P}_2\text{O}_5$  made three composts which were as follows:

- No. 1. 10 parts raw phosphate, 10 parts liquid manure.
- No. 2. 10 parts raw phosphate, 20 parts liquid manure.
- No. 3. 10 parts raw phosphate, 10 parts stable manure.

Each of these composts was mixed with 36 parts of soil. These mixtures were allowed to compost for about 15 months, and were mixed several times during the period. They were applied as fertilizers on plots. It was concluded that the phosphate was made soluble by composting, but as the check was also manured by mistake, the results obtained were not conclusive. Another experiment was tried in which horse manure was composted with raw phosphate, potassium carbonate and other salts. The results in this case were not definite.

Holdefleiss (23) composted a low grade raw phosphate rock with organic materials such as peat, humus and horse manure. He added to these liquid manure and various salts. The greatest solvent effect was brought about in a compost consisting of 216 kg. of peat, 24 kg. of rock phosphate and 48 liters of liquid manure, and in a compost composed of 216 kg. of peat, 24 kg. of rock phosphate and 5 kg. of ammonium sulfate kept moist with water. The percentages of phosphoric acid made soluble in ammonium citrate were 7.37 and 7.43, respectively.

About eleven years later Fleischer (16) in a study of the action of moorland soils on insoluble phosphate rock, found that the effect was to render a portion of the phosphoric acid soluble in water. Phosphate rock was composted with peat in the proportion of 1 to 5. One experiment was continued for an entire year. The water-soluble phosphoric acid from one compost was 5.51 per cent of the total phosphoric acid added, and the citrate-soluble was 17.08 per cent in one case. Checks containing organic matter only were not included in the experiment so that the results are of very little value.

Kissling (28) continued these investigations. His experiments were designed to study the effect of soluble salts on the solvent action of moorland soils upon phosphate rock. To 20 gm. of air-dried moorland soil there was added rock containing 0.7425 gm. of phosphoric acid. The various salts were added to this mixture. No results of any consequence were obtained. His investigations are open to the same criticism as the work of Fleischer.

Perhaps the earliest experiments conducted in the United States on composting rock phosphate with organic matter were those of Lupton

(35). In laboratory experiments he found that fermenting cottonseed meal had little solvent effect upon Florida soft phosphate rock or South Carolina ground phosphate rock as determined by the ammonium citrate method. He analyzed portions at intervals for three months. The results obtained were variable.

Pfeiffer and Thurmann (41) found a slight increase in the citrate-soluble phosphoric acid of phosphate rock when composted with peat and liquid manure, and a decided decrease in the citrate-soluble phosphoric acid of superphosphate showing that the formation of tricalcium phosphate or of organic phosphorus compounds must have taken place.

At the Ohio Experiment Station (40) a series of tests has been carried out on the value of floats and of other phosphates for reinforcing manure. In these tests floats were used at the rate of 40 pounds per ton of barnyard manure. The latter was allowed to compost for a few weeks, and was then used in plot experiments. A very slight increase in crop yield was obtained over the plots receiving uncomposted manure and floats.

Also Shutt (48) found fermenting manures to have only a slight solvent action on composted rock phosphate (apatite).

McDowell (36) composted six pounds of Tennessee rock phosphate with 228 pounds of stable manure. The floats were applied in alternate layers. After fermenting for about 13 months analyses showed no increase in either the water or citrate-soluble phosphoric acid, a condition which confirmed the results of Lupton.

Truog (53) in laboratory experiments in which floats, manure and grass were composted together, found that there was little or no solvent action of the fermenting material. In pot experiments, when the floats were thoroughly mixed with soil, favorable results were obtained, indicating that carbon dioxide given off by plant roots was instrumental in breaking down the insoluble phosphate. Where manure was added carbon dioxide production was increased; this was accompanied by a greater solvent action on the floats. He concludes that in composting experiments the dissolved substances are not removed as under field conditions; the laboratory experiments therefore fail to imitate field conditions.

Hartwell and Pember (21), in order to determine whether cow dung had any solvent action on phosphate rock, made up composts containing the two materials and allowed them to ferment for several months. The availability of the phosphate in the composts was determined by comparing the yields of millet and oats grown in duplicate pots. In summing up the results obtained they concluded, "The experiments indicate that practically no increase in the amount of available phosphorus results from mixing fresh cow dung and floats together for a number of months before applying them to the land."

The more recent experiments of Tottingham and Hoffmann (52) show that after a fermentation of 4 to 6 months the water-soluble phosphoric acid in manure and manure-floats mixture decreased. Where bac-

teria were active a saturated solution of carbon dioxide had no better action than water for extracting phosphoric acid from the mixture; but at the end of 10 months when the bacteria in the composts were practically inert it gave superior results. Acid phosphate underwent the same changes in solubility as the rock phosphate.

*Other Experiments Relating to the Availability of Insoluble Phosphates*

The solvent effect of different so-called "physiologically acid" salts on raw phosphate has been the subject of much experimental inquiry.

In 1900, Warrington (55) called attention to the fact that certain lime phosphates were made more available to plants when used together with ammonium sulfate.

Experiments carried out by Schulov (47) in 1900 and later show that ammonium sulfate and ammonium nitrate furthered the assimilation of phosphoric acid by plants from finely ground phosphorite. He ascribed this action as being due to the so-called "physiologically acid" nature of the ammonium salts. On the other hand experiments carried out with calcium nitrate showed little solvent effect on the phosphorites. He designated this salt as being "physiologically alkaline." The plants apparently assimilated the basic radicle and left the acid radicle in the soil, which would have a slight solvent effect on the phosphorite.

Other work along this line has been carried out by Kossovich (30), Prianishnikov (42), Söderbaum (50), Nedokuchaev (39), Mitscherlich and Simmermacher (37).

We may also note here Hopkins' (24) suggestion that some of the favorable results obtained with floats may be partially due to the formation of nitric acid in the course of the nitrification processes going on in the soil, which would have a soluble effect on the insoluble phosphates present.

The investigations concerning the action of decay bacteria and of other microorganisms on the mineral phosphates of the soil have not been very extensive. Work in this field has been stimulated by the knowledge that carbon dioxide is formed in large quantities in all processes of decay and putrefaction, and that the carbon dioxide thus formed when dissolved in water should hasten the solution of inert phosphates. Liebig himself pointed out (32) the probable action of carbon dioxide generated in the soil, on the solution of phosphates. Experimentally, this question was taken up by Bretschneider (6) in 1870. Indications of some solvent effect were found when phosphates were acted upon by water, and by water saturated with carbon dioxide. Similar tests were made by Karmrodt (27). The materials used by him included phosphate rock, bone ash and raw bone meal. When these materials were kept in contact with water saturated with carbon dioxide for a period of about 6 weeks a marked solvent effect was noted. In the case of the phosphate rock about 9½ per cent of the phosphoric acid had become soluble.



In 1900 Stoklasa (51) suggested that the solution of phosphoric acid in bone meal may be increased by bacteria.

Koch and Kröber (29) found that a number of insoluble phosphates were rendered soluble by the acids formed by sewage and soil bacteria from dextrose.

Sackett, Patten and Brown (45) and Brown (8) have found that certain soil bacteria exert a solvent action on raw phosphate rock, and other insoluble phosphates. They concluded that the carbon dioxide liberated in the medium by bacterial activities was the important factor, although acids formed by certain bacteria produced a slight solvent effect.

Recent investigations by Bassalik (3) show that certain bacteria have a solvent effect on apatite and other silicates. Nitrite bacteria showed an appreciable solvent action notwithstanding the slight production of acid. Butyric acid bacteria also exercised a solvent effect on apatite.

More recent investigations carried out by Hopkins and Whiting (25) corroborate the results obtained by Bassalik, in that the nitrous acid formed by nitrite bacteria was found to exert a solvent effect on pulverized rock phosphate.

Some work has also been done on the effect of iron sulfide and ferrous sulfate on insoluble phosphates. Mention may be made in this connection of the studies of Cazeneuve and Nicolle (10) who made tests on the solvent effect of ferrous sulfate on various phosphatic fertilizers, including mineral phosphate. After 8 days the mineral phosphate had suffered no change in solubility as measured by acetic acid and ammonium citrate.

Boiret and Paturel (4), using ferrous sulfate in water culture tests on plants, found that sulfuric acid was formed and the plants were consequently killed. Tests made in soils showed that the ferrous sulfate exerted no effect on the amount of phosphoric acid in the product. The ferrous sulfate in this case was not used in conjunction with other fertilizers.

Recent investigations by Galtsev and Yakushkin (18) on the combined action of phosphorite, and of iron sulfide and its oxidation products show that modified pyrite containing sulfate and sulfuric acid, produced beneficial effects on crop yields, when used in pot experiments with phosphorite, indicating a partial solution of the insoluble phosphate.

*The Oxidation of Sulfur by Microorganisms in its Relation to the Availability of Phosphates*

A series of experiments by European and American investigators on the oxidation of sulfur by microorganisms suggested to the senior author of this paper that this reaction might be of possible value in making available the phosphorus of mineral phosphates. Accordingly, experiments were planned and started in the fall of 1915. A preliminary report on these experiments was made in a recent issue of SOIL SCIENCE (34). In

the present paper the data thus far obtained are discussed at greater length. But prior to their consideration in the following pages it seems desirable for the sake of completeness to refer to certain publications which have a more or less direct bearing on the problem.

As early as 1877 it was suggested by Charles F. Panknin of Charleston, South Carolina, that sulfur when mixed with ground bone or ground mineral phosphates would be oxidized to sulfuric acid when incorporated into cultivated soil. He applied for a patent on his process in April, 1877, and succeeded in August of the same year in securing Letters Patent 193,890. In his application for the patent Panknin made the following statement: "My invention relates to a compound consisting of a mixture of finely-powdered bone or mineral phosphates and finely-powdered sulphur, the said ingredients being mixed together and applied in a dry state, and the conversion of the insoluble phosphate of lime is affected by the sulphuric acid eliminated by the natural oxidation of the sulphur of the mixture in the soil to which it is applied."

"To prepare the fertilizing compound, take 95 parts of the insoluble phosphates, (either bone or mineral phosphate,) in a finely powdered state, and mix with it 5 parts of finely-powdered sulphur, both ingredients being in a dry state, though I do not confine myself to these proportions. This mixture though only mechanical in its character, when applied to the soil, becomes slowly transformed, the sulphur being first converted into sulphuric acid by a natural process of oxidation, and when so converted, uniting with the insoluble phosphates to form a soluble phosphate, which is dissolved by the moisture of the soil, and acts in the same manner as phosphates made by artificial process."

In September, 1904, a patent application was submitted by William B. Chisholm of Charlestown, South Carolina, and Patent 824,280 was secured by him on June 26, 1906. Chisholm's invention as described by him "relates to certain new and useful improvements in fertilizers and has for its object the production of a fertilizer which in part subserves the functions of a germicide and which, while of greatly decreased cost of production in comparison with the ordinary phosphatic fertilizers now on the market, is of largely increased efficiency in use and is practically without destructive effect upon any kind of crop or of the bagging or the like in which it may be stored or transported." It is further stated by Chisholm that in the preparing of his fertilizer "the phosphate rock and the sulfur are first passed together through a preliminary crusher, reducing them to the average size of, say, a pea or bean. The crushed rough mixture is then admitted into a pulverizing mill of the Lucop or other suitable type and is reduced there in to an impalpable powder, any tailings being returned to the mill for further grinding. The resultant is an intimate and uniform admixture of phosphate rock and sulfur in the form of an almost impalpable powder and in an absolutely dry condition. I find that the percentage of sulfur, by weight, to give effective results may in some

instances be as low as forty pounds to the ton of the mixture—*i. e.*, forty pounds of sulfur to nineteen hundred and sixty pounds of phosphate rock—and in other instances from sixty to one hundred pounds of sulfur to the ton of mixture. . . . In the practical use of my invention I find that the crops produced are apparently far superior to what might reasonably be expected from the amount of available phosphate, nitrogen, and potash or other ingredients present in the final mixture. This may be due in part to the extreme fineness and intimate association of the admixed particles tending to facilitate their ready and uniform distribution through the soil, their chemical interaction under the influence of the moisture of the soil and the infiltrations of rain, and their increased solubility in said condition of impalpable powder; but I am further led to believe that the sulfur itself has an independent effect as a germicide, destroying bacterial and insect life prejudicial to the plant and either not interfering with or positively stimulating helpful bacteria.”

The above citations from the Panknin and Chisolm patents bring out some very interesting facts. It is evident that Panknin knew nearly forty years ago that sulfur when mixed with soil may be changed into sulfuric acid. Moreover he recognized the value of sulfur oxidation for transforming insoluble into soluble phosphates. He did not know, of course, that the process of sulfur oxidation is largely biological in character. Neither did he recognize that the very small quantities of sulfur recommended by him, namely one part to twenty parts of phosphate were entirely inadequate for accomplishing the desired reaction. The same criticism may be made of the process proposed by Chisolm. In both instances relatively small amounts of sulfur are directly applied to the soil with the expectation that the sulfuric acid ultimately produced would attack the insoluble phosphates. The fact was obviously overlooked by both Panknin and Chisolm that when twenty to one hundred pounds of sulfur are mixed with two to three million pounds of soil the basic substances in the latter including compounds of lime, magnesia, iron and alumina would readily react with the sulfuric acid derived from the oxidation of sulfur and no free acid would be left for any appreciable action on the insoluble phosphates. This fact was duly appreciated by the authors of this paper when the experiments described in the following pages were being planned, even though they did not know at that time, of the existence of the Panknin and Chisolm patents. It seemed to them that the inert phosphates could be best made available by means of sulfur oxidation in a relatively concentrated medium *e. g.* in a compost heap containing a relatively large proportion of both phosphate and sulfur. The data recorded below show that their reasoning was sound, as will be shown at greater length elsewhere in this paper. Meanwhile it would not be out of place to mention at least briefly the recent studies on the oxidation of sulfur by microorganisms.

In attempting to determine the value of sulfur as a fertilizer, Degruilly (11) discovered in 1911, that the sulfur applied at the rate of 109 gm. per square meter was largely oxidized to sulfates. Somewhat later Boullanger (5) was able to prove a favorable effect of sulfur on crop yields. Since the corresponding results in sterilized soil were not marked, Boullanger concluded that the oxidation of sulfur is brought about by biological activities. The results just noted were confirmed by Demolon (12) in 1912 and again in 1913 (13).

In his earlier experiments he employed 5 gm. of sulfur to each 100-gm. portion of soil. Notwithstanding these relatively large applications, much of the sulfur was oxidized to sulfuric acid. Subsequently, Demolon added 1 per cent of sulfur to garden soil and kept the mixture moist and at a temperature of 20° C. Under these conditions appreciable quantities of sulfuric acid were formed, as shown by the amounts of calcium sulfate found in the mixture at the end of 40 days. It was observed at the same time that the oxidation of sulfur was most active in a non-sterilized soil. In fertilizer experiments it was found that when sulfur was supplied in an amount equal to that of the nitrogen, the yields were as good as those obtained from a complete fertilizer. He ascribed the beneficial action of sulfur as having been due to its influence on the activities of soil bacteria, as well as to its gradual transformation into sulfuric acid and the reaction of the latter with basic material to form an increased supply of mineral plant-food.

The oxidation of sulfur was made the subject of study also by Brioux and Guerbet (7). They dealt particularly with the influence exerted on the oxidation of sulfur by the soil itself and by certain carbohydrates, peptone and other nitrogenous materials. Their investigations showed that carbohydrates had a retarding effect, while peptone and certain other nitrogenous materials accelerated it so that in 30 days 82 per cent was found to have been oxidized to sulfates. Calcium carbonate was also found to have enhanced the rate of sulfur-oxidation. They concluded, therefore, that the oxidation of sulfur in soils is a very complicated process and involves the activities of a number of species of bacteria.

Vermorel and Dantony (54), in studying the fertilizing action of sulfur and iron sulfide on wheat and beans obtained increased yields when organic matter was present. The experiment indicates that the activities of sulfifying bacteria are stimulated by organic matter.

Some interesting data concerning the influence of sulfur on soil acidity are recorded by Lint (33). In order to ascertain the rate of oxidation of sulfur in soil, 100-gm. portions of it were treated with 33 mg. of flowers of sulfur, which is an addition equivalent to 100 pounds of sulfur per acre foot of soil. The moisture content was 20 per cent. The acidity was found to increase up to the eighth week when all the sulfur was apparently oxidized. Experiments with a heavy clay loam and a sandy loam soil showed a much more rapid oxidation of the sulfur in the latter

soil. Soils with a water content of 20 per cent when allowed to dry did not cause as rapid an oxidation as those maintained at an optimum moisture content throughout the experiment.

Shedd (49) studied the oxidation of sulfur in soil and sand. With soil to which sulfur was added in amounts varying from 100 pounds to 7000 pounds per acre, he found by analysis, that 60 to 80 per cent of the sulfur, regardless of the amount added was oxidized after a period of about 4 months. Sulfur was oxidized also in the sand but not to the extent that it was in the soil.

Kappen and Quensell (26) made a study of the sulfur cycle in soils. In connection with these experiments they studied the oxidation of ground rhombic sulfur and the so-called "Schwefel milch." The latter was prepared by mixing together 2 parts of sulfur, 1 part of calcium hydroxide and 13 parts of water. The solution was heated until the constituents dissolved. After diluting and precipitating with dilute hydrochloric acid, the precipitated sulfur was filtered and washed. Two hundred mg. of the dry sulfur thus prepared and of the ground rhombic sulfur were mixed with the different soils under investigation, incubated for six weeks, extracted with 10 per cent of hydrochloric acid and the sulfates determined in the extract. The results which they obtained are quoted below to show the striking oxidation of the precipitated sulfur.

Soil	SO <sub>4</sub> from 200 mg. of Sulfur	
	Rhombic Sulfur	Precipitated Sulfur
Glass Sand .....	3.69 mg.	44.63 mg.
Sandy Soil .....	3.81 mg.	144.22 mg.
Loam Soil .....	16.44 mg.	227.57 mg.
Meadow Soil .....	10.91 mg.	284.01 mg.

Brown and Kellogg (9) report some experiments on the so-called sulfifying power of soils. They find that each soil has a definite sulfifying power. Organic matter was found to increase the amount of sulfur oxidized. Manure and green manure increased it up to a certain point. Moisture was found to play an important part in the oxidation process, and the optimum moisture condition was apparently 50 per cent of complete saturation. Other experiments which they carried out show that aeration is an important factor, as is also the absence of carbohydrates. The presence of bacteria is essential, although the production of a small amount sulfates was brought about by chemical action.

Ames and Boltz (2) in their recent study of the effect of sulfur on the growth of plants, found that it produced increased yields when applied to certain crops. In order to determine whether or not this beneficial action was due to the solvent action on the mineral soil constituents of the acids produced during the sulfifying processes, they conducted a composting experiment in which sterilized and unsterilized soils, a loam and a clay soil, and pure quartz sand were used. To 500-gm. portions of the soil and sand were added 0.25 gm. of flowers of sulfur and Tennessee phosphate

rock in amounts containing 0.06 gm. of phosphorus. To each mixture was added 400 c.c. of distilled water. After 2 months they found but a slight increase in the availability of the phosphorous, as measured by 0.2 per cent hydrochloric acid. The acidity of the mixture had also increased but little.

These investigators have evidently assumed that sulfofication is a process brought about under anaerobic conditions; while the experiments of Brown and Kellogg (9) and those of Lipman, McLean and Lint (34) show that aerobic conditions favor the most rapid oxidation of sulfur in the soil.

## EXPERIMENTAL

### *Methods*

*Composts.* In making up the composts 100-gm. portions of soil were placed in tumblers, the phosphate rock and sulfur added together with other materials, if such were used in the experiment, and the whole thoroughly mixed. In all cases the composts were arranged in duplicate and duplicate analyses made. Moisture was then added and the tumblers were covered with petri dishes and placed in a dark closet kept at a temperature of 22° to 24° C. The different composts were weighed and water was added up to the optimum every two weeks.

*Sampling.* In order to obviate the need of making moisture determinations for each analysis, the entire amount of soil from each tumbler was placed upon the scale pan, weighed and one-fifth of the total weight taken for each determination.

*Ammonium-Citrate-Soluble Phosphoric Acid.* The sample of compost was transferred to a 500 c.c. volumetric flask with 100 c.c. of neutral ammonium citrate solution which had been heated to 65° C. The extraction was made for 30 minutes in a water bath kept at a temperature of 65° C. The flasks were cooled and made up to volume with distilled water. After thorough shaking, the extract was filtered and an aliquot representing 1/50 of the entire compost taken for analysis. This aliquot was transferred to a 400-c.c. beaker and evaporated to dryness with aqua regia several times, in order to oxidize the organic matter, and to render insoluble any silica which might be in solution. The residue was taken up with acid and the phosphoric acid determined in the usual manner by the volumetric method.

*Water-Soluble Phosphoric Acid.* The sample of compost taken for analysis was transferred to a filter with hot water, and thoroughly washed. The washing was repeated until the filtrate measured 500 c.c. After cooling, making up to volume and vigorously shaking, an aliquot representing 2/25 of the total compost was transferred to a 250-c.c. beaker, a few drops of nitric acid were added, the contents of the beaker were evaporated to a volume of 25 c.c., and the phosphoric acid determined in the usual manner.

*Acidity (Relative).* For determining the relative acidity the following method was used. One-fifth of the compost from each tumbler was transferred to a 200-c.c. volumetric flask with 160 c.c. of hot distilled water, thoroughly shaken every five minutes throughout a period of one-half hour, after which the contents of the flasks were cooled, made up to volume and thoroughly shaken. An aliquot was drawn off, boiled for 5 minutes to drive off any carbon dioxide which might be present, phenolphthalein was added and the liquid titrated to a faint pink color with N/50 KOH.

*Sulfates.* Five-gm. portions of the composts were transferred to flasks, with 100 c.c. of 1 per cent hydrochloric acid, placed on a shaking machine and shaken for 7 hours. The sulfates were then determined in aliquots by the usual method.

*Oxidation of Sulfur in Different Soils as Affecting the Availability of Rock Phosphate*

Since different soils have been found by Brown and Kellogg (9) and by Kappen and Quensell (26) to show differences in their ability to oxidize sulfur, this experiment provides for the use of two soils and of sea sand. One of these, a greenhouse soil, was a red silt loam soil to which had been added composted manure. It contained .2970 per cent of  $P_2O_5$ , 10.14 per cent of  $Fe_2O_3$  and  $Al_2O_3$ , and 3.04 per cent of organic carbon. The other, a Sassafra soil contained 1.07 per cent of organic carbon, 5.39 per cent of  $Fe_2O_3$  and  $Al_2O_3$  and 0.1950 per cent of  $P_2O_5$ . The soils were air dried and, for the sake of uniformity, passed through a 10-mesh sieve. The phosphate rock, or floats, used in this experiment was Tennessee brown rock phosphate containing 30 per cent of total  $P_2O_5$  and ground so that 95 per cent would pass through a sieve with 10,000 meshes to the square inch. The 15-gm. portion of the rock phosphate used in each compost contained 4500 mg. of  $P_2O_5$ . Two forms of sulfur were employed: flowers of sulfur and precipitated sulfur; the latter was prepared according to the method of Kappen and Quensell (26).

The composts of soil, rock phosphate and sulfur were made according to the plan given, transferred to tumblers, thoroughly mixed, and water added in amounts equivalent to 50 per cent of the water-holding capacity. In order to insure proper inoculation and to supply mineral food to the bacteria in the sand mixtures, 10 c.c. of the water were added in the form of soil infusion, obtained by shaking for 10 minutes 100 gm. fertile soil with 200 c.c. of a dilute synthetic culture medium containing no phosphorus. All the tumblers were then weighed and the weights recorded on each, so that any water lost by evaporation could be replaced. They were then covered with petri dishes and placed in a dark closet, which was kept at a temperature of 22° to 24° C.

For the study of the weekly changes in the various composts, analyses were made at the beginning of the experiment and at the end of each week, up to the eighth week; after that the analyses were made at the end of every two weeks up to the twelfth week, after which the composts were allowed to stand for a longer period. The water-soluble phosphoric acid in the mixtures was determined at different intervals. The weekly changes in the citrate-soluble phosphoric acid and acidity of the composts are recorded in Tables I, II and III. In figure 1 the results are shown graphically.

#### Plan of Experiment

Several sets of this experiment were arranged in duplicate according to the following plan.

#### TREATMENT

- |          |  |
|----------|--|
| Lab. No. | (Optimum moisture added to each)                 |
| 1.       | 100 gm. Sea Sand, inoculated with soil infusion. |
| 2.       | Same as No. 1., 5 gm. Flowers of Sulfur added.   |
| 3.       | Same as No. 1., 15 gm. Floats added.             |
| 4.       | Same as No. 3., 5 gm. Flowers of Sulfur added.   |
| 5.       | 100 gm. Greenhouse Soil.                         |
| 6.       | Same as No. 5, 5 gm. Flowers of Sulfur added.    |
| 7.       | Same as No. 5, 15 gm. Floats added.              |
| 8.       | Same as No. 7, 5 gm. Flowers of Sulfur added.    |
| 9.       | 100 gm. Sandy Loam Soil.                         |
| 10.      | Same as No. 9, 5 gm. Flowers of Sulfur added.    |
| 11.      | Same as No. 9, 15 gm. Floats added.              |
| 12.      | Same as No. 11, 5 gm. Flowers of Sulfur added.   |
| 13.      | Same as No. 9, 5 gm. Precipitated Sulfur added.  |
| 14.      | Same as No. 13, 15 gm. Floats added.             |

The data included in Tables I, II and III show that as a result of the oxidation of the sulfur there was not only an increase in the acidity of certain of the mixtures but also an accumulation of water-soluble and citrate-soluble phosphoric acid when the sulfur and tricalcic phosphate were used together.

A careful comparison of the results recorded in these tables will show some very definite differences as brought about by treatment, as well as by variation in the physical and chemical properties of the soils used. It will be noted, among other matters, that in the sand mixtures there was practically no change in the amounts of acid and of available phosphorus when neither sulfur nor tricalcic phosphate was employed. The initial acidity was here equivalent to 6 c.c. of N/50 KOH. At the end of the third week there was a slight increase to an equivalent to 8 c.c. After that there were important fluctuations to the end of the thirtieth week when the amount found was equivalent to 7 c.c. of N/50 KOH. Similarly,



but slight changes occurred in these mixtures in the content of available phosphoric acid. The greatest variation was found at the end of the seventh week, when 25.39 mg. of citrate-soluble phosphoric acid were present, as against 34.58 mg. at the beginning of the experiment. The decrease in this instance was probably due to the assimilation of a portion of the available phosphorus by soil microorganisms.

The sand mixtures which received additions of sulfur alone showed a progressive increase in acidity until the end of the eighteenth week. Lack of material prevented the making of corresponding determinations at the end of the twenty-fourth and thirtieth weeks. It will be observed,

TABLE I  
SAND: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions							
	None		5 gm. Flowers of Sulfur		15 gm. Floats		5 gm. Fl. of Sulfur 15 gm. Floats	
	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.
Beginning .....	6.00	34.58	7.25	35.06	5.75	136.62	7.50	139.04
End of 1st week....	6.00	33.85	12.00	35.06	6.00	169.26	9.50	171.68
End of 2nd week....	.....	.....	.....	.....	7.25	139.00	21.50	180.38
End of 3rd week....	8.00	32.64	34.50	33.37	7.50	177.72	46.50	160.80
End of 4th week....	.....	.....	.....	.....	8.25	187.39	143.00	234.55
End of 5th week....	6.50	27.81	41.50	28.23	6.50	168.05	174.50	224.82
End of 6th week....	.....	.....	.....	.....	7.75	122.10	177.00	272.02
End of 7th week....	7.50	25.39	58.00	24.18	9.50	136.62	215.00	258.73
End of 8th week....	.....	.....	.....	.....	7.00	136.70	208.00	262.87
End of 10th week....	7.50	31.43	96.00	34.52	7.50	136.62	338.00	337.31
End of 12th week....	.....	.....	96.00	.....	7.00	138.20	336.00	390.62
End of 15th week....	.....	.....	100.00	33.80	6.85	116.47	328.50	400.68
End of 18th week....	7.20	27.90	100.00	28.90	7.10	140.20	414.00	455.95
End of 24th week....	.....	.....	.....	.....	7.30	127.80	932.00	949.07
End of 30th week....	7.00	30.82	.....	.....	7.10	129.30	976.00	1115.10
Increase .....	.....	.....	.....	.....	.....	.....	968.50	966.06

nevertheless, that there was here an increase from an initial acidity equivalent to 7.25 c.c. of N/50 KOH to an equivalent 100 c.c. of N/50 KOH. It is safe to assume, therefore, that notwithstanding the poverty of the culture medium in nitrogen and phosphorus compounds the sulfifying organisms multiplied sufficiently to convert an appreciable amount of sulfur into sulfuric acid. On the other hand, the citrate-soluble phosphoric acid remained practically constant in amount. Obviously the small quantities of sulfuric acid that had accumulated in the sand portions found no phosphates to act on. Thus at the end of the eighteenth week the sulfur-sand mixtures contained an equivalent of 28.90 c.c. of N/50 KOH, as against 27.90 c.c. N/50 KOH, in the sand alone.

In the floats-sand mixtures there were insignificant changes in the acidity of the medium from the first to the thirtieth week. Moreover, there was a limited increase followed by a decrease in the amount of citrate-soluble phosphoric acid. The initial content of citrate-soluble phosphoric acid was, in these mixtures, 136.62 mg. At the end of the fourth week it was 187.39 mg. and at the end of the thirtieth week 129.30 mg. In this case the absence of acid prevented the accumulation of soluble phosphates beyond the amount originally present in the 15 gm. of floats.

In the sulfur-floats-sand mixtures the conditions were different from those in the sand, the sulfur-sand or the floats-sand mixtures. Here the presence of tricalcic phosphate favored the development of the sulfocy-

TABLE II  
GREENHOUSE SOIL: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions							
	None		5 gm. Flowers of Sulfur		15 gm. Floats		5 gm. Fl. of Sulfur 15 gm. Floats	
	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity c.c. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.
Beginning .....	2.50	111.95	5.00	105.18	4.25	143.39	5.50	142.66
End of 1st week....	2.00	111.71	8.75	96.72	4.25	166.84	11.50	166.84
End of 2nd week....	.....	.....	.....	.....	3.00	176.51	18.75	203.11
End of 3rd week....	4.75	122.11	42.75	120.90	6.25	191.02	89.00	272.75
End of 4th week....	.....	.....	.....	.....	6.00	206.74	224.00	227.29
End of 5th week....	2.75	79.79	137.00	100.34	2.25	166.13	209.00	230.92
End of 6th week....	.....	.....	.....	.....	6.00	148.21	281.00	258.73
End of 7th week....	5.25	83.42	310.60	103.49	6.00	157.17	324.00	253.89
End of 8th week....	.....	.....	.....	.....	5.50	160.79	352.00	320.38
End of 10th week....	6.50	105.18	4404.00	154.75	5.50	153.54	646.00	310.94
End of 12th week....	.....	.....	4560.00	.....	.....	.....	596.00	396.88
End of 15th week....	.....	.....	4700.00	160.10	4.96	138.77	710.00	1982.40
End of 18th week....	3.98	100.00	4690.00	150.20	6.00	149.62	2348.00	2605.62
End of 24th week....	.....	.....	.....	.....	.....	.....	2402.00	3840.90
End of 30th week....	6.00	87.90	.....	.....	5.13	160.40	2400.00	4126.48
Increase .....	.....	.....	.....	.....	.....	.....	2394.50	3983.82

ing organisms by supplying the element phosphorus essential for their development, and, perhaps, also by preventing the accumulation of too great an amount of free acid in the moisture films surrounding the sand grains. To avoid misunderstanding it should be added here that the amounts of acid as shown by titration of the sulfur-floats-sand mixtures were probably due to acid salts rather than free sulfuric acid.

It is worth while to note here that the increase in the acidity equivalents was striking and suggestive. At the end of the third week the acidity found was equivalent to 46.50 c.c. of N/50 KOH. In the

TABLE III  
SASSAFRAS LOAM SOIL: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions									
	None		5 gm. Flowers of Sulfur		15 gm. Floats		5 gm. Flowers of Sulfur 15 gm. Floats		5 gm. Precipitated Sulfur,	
	Activity cc N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Activity cc N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Activity cc N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Activity cc N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Activity cc N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.
Beginning	6.00	79.79	6.50	83.42	5.55	155.96	7.50	154.27	8.75	143.87
End of 1st week	9.50	77.37	11.25	94.30	10.00	162.01	10.00	164.91	14.30	169.26
End of 2nd week	.....	.....	.....	.....	10.00	191.01	11.50	222.46	.....	.....
End of 3rd week	6.00	120.90	33.50	125.74	6.25	171.68	107.50	209.16	130.25	247.85
End of 4th week	.....	.....	.....	.....	7.00	180.14	360.50	269.61	.....	.....
End of 5th week	7.50	99.14	450.50	103.97	5.25	165.63	353.00	342.15	378.50	264.77
End of 6th week	.....	.....	.....	.....	6.50	155.75	421.00	406.22	.....	.....
End of 7th week	7.00	71.33	597.00	89.47	10.50	158.38	485.00	339.73	417.50	425.57
End of 8th week	.....	.....	.....	.....	8.50	175.67	478.00	508.98	465.00	303.46
End of 10th week	7.00	96.72	1240.00	100.35	8.50	158.86	660.00	518.66	475.00	510.92
End of 12th week	.....	.....	1300.00	.....	.....	.....	637.00	655.20	604.00	535.59
End of 15th week	.....	.....	1440.00	101.40	7.45	168.50	570.25	867.30	600.25	597.20
End of 18th week	6.95	80.20	1590.00	100.00	7.50	161.30	530.00	612.07	604.00	670.30
End of 24th week	.....	.....	.....	.....	.....	.....	534.00	688.88	576.00	966.42
End of 30th week	7.10	75.15	.....	.....	6.70	170.81	690.00	822.70	726.00	662.87
Increase	.....	.....	.....	.....	.....	.....	682.50	668.43	717.25	639.18

sulfur-sand mixture the corresponding equivalent was only 34.50 c.c. N/50 KOH. At the end of the fifth week the acidity equivalents in the sulfur-sand and sulfur-floats-sand mixtures were 41.50 c.c. and

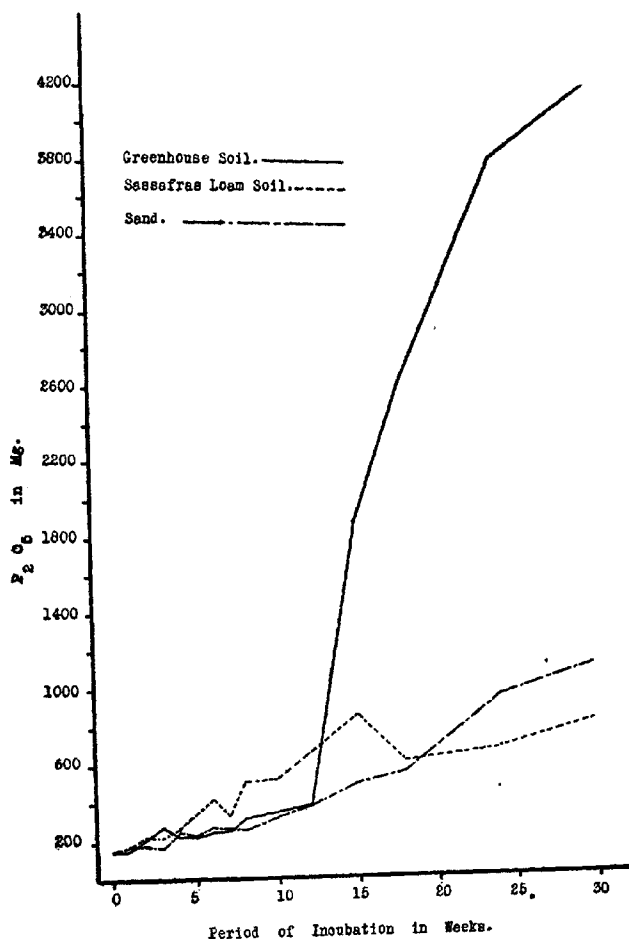


Fig. 1.—Curves showing the citrate-soluble phosphoric acid in the composts during a period of 30 weeks.

174.50 c.c., respectively. At the end of the eighteenth week the corresponding figures were 100.00 c.c. and 414.00 c.c., respectively. Be-

tween the eighteenth and the end of the twenty-fourth week the acidity equivalent of the sulfur-floats-sand mixtures increased from 414.00 c.c. to 932.00 c.c. It would seem, therefore, that the sulfonation process gained in intensity most markedly after the eighteenth week. This assumption is supported by the data representing the amounts of citrate-soluble phosphoric acid found at the end of the different periods.

Comparing the figures for citrate-soluble phosphoric acid in the floats-sand and sulfur-floats-sand mixtures, one may note relatively small differences for the first three weeks. Beyond that period the differences become marked in favor of the sulfur-floats-sand mixtures. After the fifth week there was a decrease in the amounts of citrate-soluble phosphoric acid in the floats-sand mixtures, whereas in the sulfur-floats-sand mixtures there was, with one exception, a constant increase. It will be observed, for instance, that at the end of the eighteenth week there was found in the floats-sand mixture an equivalent of 140.20 mg. of citrate-soluble phosphoric acid, while in the sulfur-floats-sand mixture the corresponding amount was 455.95 mg. At the end of the twenty-fourth week the corresponding figures were 127.80 mg. and 949.07mg., respectively; and at the end of the thirty weeks, 129.30 mg. and 1115.10 mg., respectively. There can be no doubt therefore, that, notwithstanding the poverty of the sand as a culture medium, a large proportion of the sulfur added was oxidized to sulfuric acid, and that the latter readily reacted with the tricalcic phosphate to form citrate-soluble phosphate.

As a culture medium the greenhouse soil was much superior to the sand. It was originally rich in nitrogen and mineral plant-food constituents and had been still further enriched by additions of organic matter. The latter had undergone more or less thorough decomposition prior to the use of the soil in the experiments described here. Hence the sulfifying bacteria should have accomplished a greater amount of change in the greenhouse soil than they did in the sand, as is indeed borne out by the data presented in Table II.

It was to be expected that the soil portions which received no addition of either sulfur or floats would show no appreciable increase in acidity or available phosphoric acid. Such was really the case as indicated by the figures in columns 1 and 2 of Table II. There was, as a matter of fact, a slight decrease in the amount of available phosphoric acid, brought about, probably, by the assimilation of some of the available phosphorus by the microorganisms in the soil. On the other hand, in the sulfur-soil mixtures there was a steady and marked increase in the acidity equivalents until the end of the seventh week. At that time the amount of acid found was equivalent to 310.60 c.c. of N/50 KOH. But from the end of the seventh to the end of the tenth week there was a most remarkable increase in the acidity equivalent. From an acidity equivalent of 310.60 c.c. there was an increase to an acidity equivalent of 4404.00 c.c. of N/50 KOH. After that there was a slight,

but nevertheless, marked increase until the end of the eighteenth week. Corresponding soil portions were not available for the determination of the acidity equivalents at the end of the twenty-fourth and thirtieth weeks. With the accumulation of free acid and of acid salts in the soil portions there was also an increase in the amount of available phosphoric acid. Evidently the phosphorus compounds in the soil were acted upon by the sulfuric acid and the amount of available phosphoric acid increased from 105.18 mg. to a maximum of 160.10 mg. at the end of the eighteenth week.

In the floats-soil mixtures there was no perceptible accumulation of free acid or of available phosphates. In the absence of sulfur this could not have taken place. But in the sulfur-floats-soil mixtures the results were quite different. Here the sulfur led to an accumulation of acid which, for a time, was greater in quantity than in the corresponding sulfur-soil portions. It will be noted, for instance, that at the end of the fifth week the acidity equivalents in the sulfur-soil and sulfur-floats-soil mixtures were 137.00 c.c., and 209.00 c.c., respectively. However, after the eighth week the increase in the acidity equivalents was much slighter in the sulfur-floats-soil mixtures. At the end of the tenth week the acidity equivalent of the latter was only 646.00 c.c. as against 4404.00 c.c. in the sulfur-soil mixtures. A greater increase in the acidity equivalents of the sulfur-floats-soil mixtures occurred between the eighteenth and the twenty-fourth weeks; nevertheless, the maximum accumulation of acid was never as great as it was in the corresponding sulfur-soil mixtures.

It must be concluded, therefore that much of the acid which resulted from the oxidation of the sulfur in the sulfur-floats-soil mixtures had reacted with the tricalcic phosphate and made its phosphorus available as measured by the method of the Official Agricultural Chemists. Proof of this assumption is furnished in the last column of Table II. It is shown there that the accumulation of available phosphoric acid proceeded at first rather slowly. At the end of the twelfth week the amount of available phosphoric acid was equivalent to only 396.88 mg. After that time, however, the increase was very rapid, for the available phosphoric acid was equivalent to 1982.40 mg. at the end of the fifteenth week, 2605.62 mg. at the end of the eighteenth week, 3840.90 mg. at the end of the twenty-fourth week and 4126.48 mg. at the end of the thirtieth week.

The amounts of available phosphoric acid shown to have been present by the official method were probably below the true value, since some of the phosphoric acid had entered into combination with iron and aluminum compounds whose availability to growing crops may be greater than that indicated by the official method. As it is, more than 85 per cent of the phosphoric acid present in the 15 gm. of floats had become available. Hence it is safe to assume that in suitable soil cul-

ture media large quantities of sulfur may be oxidized by microorganisms; and that the sulfuric acid accumulating in the soil may react with insoluble tricalcic phosphates. That the nature of the medium is one of the controlling factors is readily seen from a comparison of the sand and soil mixtures, respectively, to which both sulfur and floats had been added. The data in question which may be found in the last columns of Table I and II show that at the end of the twelfth week the sand mixtures contained 390.62 mg. of available phosphoric acid while the corresponding greenhouse soil mixtures contained 396.88 mg. But at the end of the fifteenth week the corresponding figures were 400.68 mg. and 1982.40 mg., respectively; at the end of the twenty-fourth week, 949.07 mg. and 3840.90 mg., respectively; and at the end of the thirtieth week 1115.10 mg. and 4126.48 mg., respectively.

In Table III are given the results obtained in the Sassafras loam soil. These results differ in degree rather than in kind, from those already recorded for the sand and for the greenhouse soils. A brief survey of the data should therefore suffice to confirm those already discussed. The same absence of acid and of available phosphorus accumulation in the soil (alone) portion, and the same marked acid accumulation in the sulfur-soil portions may be noted. In the case of the sassafras soil the acid accumulation was not as great as in the greenhouse soil, but much greater than in the sand. Similarly there was no accumulation of available phosphoric acid in the floats-soil portions. Curiously enough, the accumulation of available phosphoric acid in the sulfur-floats-soil mixtures was greater in the sassafras soil at the end of the tenth week than it was in the corresponding sand and greenhouse soil portions. Subsequently, however, both the sand and greenhouse soil portions showed a great accumulation of available phosphoric acid. Thus at the end of thirty weeks the figures for the sand, greenhouse soil and sassafras soil were 1115.10 mg., 4126.48 mg., and 822.70 mg., respectively. There is no doubt that the failure of the sassafras soil to accumulate larger quantities of available phosphoric acid should be ascribed partly to the less favorable conditions which it offered for rapid oxidation. It is possible, also, that the chemical constitution of this soil may have had something to do with the differences noted. It will be observed, further, that in the soil portions in which precipitated sulfur was used in the place of flowers of sulfur the results obtained were very much like those already considered. They serve to show that the biological changes were limited in a rather definite way by the physical and chemical properties of the medium. They also serve to confirm the accuracy of the analytical determinations.

It may not be out of place here to point out again that the wide variation in the composition of the soils employed in these experiments and the consequent differences in their absorptive power must have

played a part in the accumulation both of acid and of available phosphoric acid. As has been shown by Fraps (17) the absorptive power of soils is closely related to their iron and aluminum content. It is probable that a greater proportion of the phosphorus of the floats was made available than is actually shown by the ammonium citrate extractions.

The absorptive power of the sand and the two soils was determined by adding to 50 gm. of each, 100 c.c. of a solution containing 300 mg. of  $P_2O_5$  in the form of potassium phosphate. After one week the solutions were filtered off from the soil and the amount of phosphorus still in solution determined. From these determinations the amount of  $P_2O_5$  fixed per gram of soil was calculated. The results are as follows:

Sand .....	0.354 mg. $P_2O_5$ fixed per gram
Greenhouse soil .....	4.000 mg. $P_2O_5$ fixed per gram
Loam soil .....	2.650 mg. $P_2O_5$ fixed per gram

From the results of the fixation tests it can be seen that the greenhouse soil was able to fix about double the amount of  $P_2O_5$  fixed in the Sassafras loam soil. It must be assumed therefore, that a portion of the phosphoric acid liberated from the floats was again made insoluble by combining with the iron and aluminum present. The amounts of available phosphoric acid shown by the ammonium citrate extractions must therefore differ, somewhat, from the amounts actually made available.

#### The Accumulation of Water-Soluble Phosphoric Acid

The water-soluble phosphoric acid found in the different soil mixtures was determined and its amount recorded in Table IV.

The results recorded in Table IV are the averages of duplicate determinations. It will be noted that the content of water-soluble phosphoric acid increased in all of the mixtures which had received additions of both sulfur and floats. A slight increase is shown in the sassafras and greenhouse soil portions which had received additions of floats alone, but this increase is within the limits of experimental error.

At the end of the eighth week the sulfur-floats-sand mixtures contained 100.95 mg. of water-soluble phosphoric acid. The corresponding amounts in the greenhouse and sassafras soils were 54.71 mg. and 128.15 mg., respectively. It seems, therefore, that whatever phosphate had been made water-soluble in the greenhouse soil by the oxidized sulfur was changed again into water-insoluble forms. The same relations appear at the end of the tenth and twelfth weeks. At the end of the fifteenth week the content of water-soluble phosphoric acid in the sulfur-floats-sand, sulfur-floats-greenhouse soil and the sulfur-floats-sassafras soil was 185.23 mg., 999.56 mg. and 178.11 mg., respectively. It is evident that the greenhouse soil had gained in a very striking way on the other



TABLE IV  
THE CONTENT OF WATER-SOLUBLE PHOSPHORIC ACID

	Beginning $P_2O_5$ , mg.	End of 8th Week $P_2O_5$ , mg.	End of 10th Week $P_2O_5$ , mg.	End of 12th Week $P_2O_5$ , mg.	End of 15th Week $P_2O_5$ , mg.	End of 18th Week $P_2O_5$ , mg.	End of 24th Week $P_2O_5$ , mg.	End of 30th Week $P_2O_5$ , mg.	Increase Due to Sulfur $P_2O_5$ , mg.
100 gm. Sand, 15 gm. Floats .....	17.72	9.07	12.39	.....	15.10	14.10	.....	.....	.....
100 gm. Sand, 15 gm. Floats, 5 gm. Flowers of Sulfur .....	17.09	100.95	156.02	174.03	185.23	230.45	473.30	557.55	540.46
100 gm. Greenhouse Soil, 15 gm. Floats .....	21.28	32.64	30.38	.....	30.10	28.78	.....	.....	.....
100 gm. Greenhouse Soil, 15 gm. Floats, 5 gm. Flowers of Sulfur...	19.91	54.71	44.58	55.71	999.56	1295.99	1234.04	1437.24	1417.33
100 gm. Sandy Loam Soil, 15 gm. Floats .....	20.35	10.27	25.24	.....	18.40	20.13	.....	.....	.....
100 gm. Sandy Loam Soil, 15 gm. Floats, 5 gm. Flowers of Sulfur...	20.97	128.15	129.97	136.78	178.11	251.88	215.23	224.26	203.29
100 gm. Sandy Loam Soil, 15 gm. Floats, 5 gm. Precipitated Sulfur...	17.52	128.76	121.20	113.93	118.32	200.46	218.94	223.02	205.50

soils. The differences are even more striking at the end of the eighteenth and thirtieth weeks. At the end of the twenty-fourth week the greenhouse soil failed for some reason to show an increase over the eighteenth week. On the whole, the data at hand show an interesting accumulation of water-soluble phosphoric acid in the sand and greenhouse soils at the end of the thirtieth week. A comparison of Table IV with Tables I and II will show that in the sand more than half of the available phosphoric acid was present in water-soluble form at the end of the thirty weeks. In the greenhouse soil more than a third of the available phosphoric acid was water-soluble at the end of thirty weeks. In this instance, again, the mechanical and chemical nature of the soil media employed served to determine the extent of absorption and adsorption, and hence the proportion of citrate-soluble and water-soluble phosphoric acid. As to the relative influence of the flowers of sulfur and precipitated sulfur used with the safflower soil, the determinations agree fairly well and do not show that the precipitated sulfur was superior to the flowers of sulfur.

#### The Oxidation of Sulfur as Measured in Terms of Sulfates

The determination of the yields of sulfates in the various soil mixtures shows clearly to what extent the sulfur was oxidized at the different periods. It will be noted from the data shown in Table V that, in the sand to which sulfur alone was added, the initial sulfur content, expressed in terms of  $\text{SO}_4$ , was 6 mg. At the end of the fifth week, the corresponding sulfur content was 15.56 mg. A gradual increase occurred to the end of the eighteenth week. No determinations were made after that period in the sulfur-sand mixtures for lack of material.

We note, further, that, in the sand to which both sulfur and floats were added, the oxidation of the sulfur was much more rapid than it was in the sulfur-sand mixtures. Thus, at the end of the fifth week, the sulfur-floats-sand mixtures contained 612.39 mg. of  $\text{SO}_4$ , as against only 15.56 mg. in the sulfur-sand mixtures. At the end of the fifteenth week, the corresponding figures were 908.64 as against 74.33, and, at the end of the eighteenth week, 1102.08 as against 100.25. At the end of the thirtieth week, there were found in the sulfur-floats-sand mixtures 2603.62 mg. of  $\text{SO}_4$ , equivalent to 816.78 mg. of sulfur. It is clear, therefore, that the presence of tricalcium phosphate hastened the oxidation of the sulfur, probably for two reasons. It may be assumed that the microorganisms responsible for the oxidation of the sulfur require more or less phosphorus for their life activities. It may be assumed, also, that, as the sulfur is oxidized to sulfuric acid, the latter may accumulate in sufficient quantity to make the medium too acid for the proper development of the microorganisms. By neutralizing some of the free acid, the tricalcic phosphate reduces the acidity of the medium and makes possible thereby the continued growth of the sulfur-oxidizing

organisms. That this assumption is justified is borne out by the figures given in Tables I, II and III, showing the amounts of phosphorus available according to the methods of the Official Agricultural Chemists. The data in question are again recorded in Table V. It is shown there that the phosphoric acid soluble in ammonium citrate was equivalent in the sulfur-floats-sand mixtures to 85.78 mg. at the end of the fifth week,

TABLE V  
THE CONTENT OF SULFATES IN THE DIFFERENT MIXTURES

Time	Additions						
	5 gm. Sulfur				5 gm. Sulfur, 15 gm. Floats		
	P <sub>2</sub> O <sub>5</sub> Soluble in Ammonium Citrate mg.	Sulfates Soluble in 1% HCl		Amount of Sulfur Oxidized mg. of S	P <sub>2</sub> O <sub>5</sub> Soluble in Ammonium Citrate Inc. over Check mg.	Sulfates Soluble in 1% HCl	
		Average mg. of SO <sub>4</sub>	Inc. over Check mg. of SO <sub>4</sub>			Average mg. of SO <sub>4</sub>	Inc. over Check mg. of SO <sub>4</sub>
							Amount of Sulfur Oxidized mg. of S
SAND							
Beginning .....	35.06	6.00	.....	.....	.....	156.70	.....
End of 5th week..	28.23	15.56	9.56	3.19	85.78	612.39	455.69
End of 7th week..	24.18	67.41	61.41	20.50	119.69	699.31	542.61
End of 15th week..	33.80	74.33	68.33	22.81	261.64	908.64	851.94
End of 18th week..	28.90	100.25	94.25	31.46	316.91	1102.08	945.38
End of 24th week..	.....	.....	.....	.....	810.03	2431.78	2275.08
End of 30th week..	.....	.....	.....	.....	976.06	2603.62	2446.92
GREENHOUSE SOIL							
Beginning .....	105.18	121.10	.....	.....	.....	152.16	.....
End of 5th week..	100.34	981.79	860.69	287.27	88.26	1424.30	1272.14
End of 7th week..	103.49	1559.12	1438.02	480.01	111.23	2007.03	1854.87
End of 15th week..	160.10	5294.16	5173.06	1727.75	1839.74	5744.64	5592.48
End of 18th week..	150.20	5873.14	5752.04	1920.02	2462.96	8559.60	8407.44
End of 24th week..	.....	.....	.....	.....	3698.24	9213.60	9061.44
End of 30th week..	.....	.....	.....	.....	3983.82	10524.96	10372.80
SASSAFRAS LOAM							
Beginning .....	83.42	130.25	.....	.....	.....	168.10	.....
End of 5th week..	103.97	708.54	578.29	193.03	187.88	1035.36	867.26
End of 7th week..	89.47	751.88	621.63	207.49	185.46	993.70	825.60
End of 15th week..	101.40	1116.61	986.36	329.25	713.03	2706.24	2538.14
End of 18th week..	100.00	1381.11	1250.86	417.52	457.80	2027.04	1858.94
End of 24th week..	.....	.....	.....	.....	534.61	2224.37	2056.27
End of 30th week..	.....	.....	.....	.....	668.43	2477.18	2309.08

to 261.64 mg. at the end of the fifteenth week, to 810.03 mg. at the end of the twenty-fourth week, and to 976.06 mg. at the end of the thirtieth week.

In the greenhouse soil mixtures, the corresponding reactions were very similar in character. Because of its more favorable character as a medium for the growth of the microorganisms, the oxidation of the

sulfur proceeded more rapidly in it. We note, thus, that the content of sulfates, expressed in terms of  $\text{SO}_4$ , in the sulfur-soil mixtures was 981.99 mg. at the end of the fifth week, 5294.16 mg. at the end of the fifteenth week, and 5873.14 mg. at the end of the eighteenth week. In the corresponding sulfur-floats-soil mixture, the oxidation of the sulfur was again more rapid than it was in the sulfur-soil mixtures, as was already noted in the case of the sand medium. For instance, at the end of the fifth week, the sulfur-floats-soil mixtures contained an equivalent of 1424.30 mg. of  $\text{SO}_4$  as against 981.79 mg. of  $\text{SO}_4$  in the sulfur-soil mixtures. Similar differences were found at the end of the other

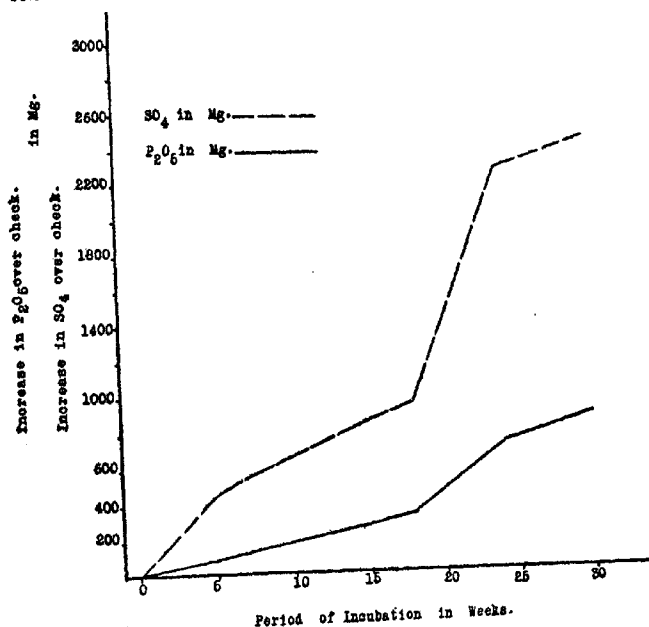


Fig. 2.—Curves showing the citrate-soluble  $\text{P}_2\text{O}_5$  and  $\text{SO}_4$  found in the composts made up with the sand during a period of 30 weeks.

periods. It may be noted particularly that, at the end of the thirtieth week, the sulfur-floats-soil mixtures contained an equivalent of 10,524.96 mg. of  $\text{SO}_4$ , which would correspond to 3462.44 mg. of sulfur. It seems, therefore, that, in the greenhouse soil, nearly 3.5 gm. of sulfur were oxidized to sulfates out of a total of 5 gm. of sulfur applied. This change was accomplished in the mixtures containing both floats and sulfur and in a period of thirty weeks. As the amount of oxidized sulfur increased in these mixtures, the amount of available phosphorus also increased. This should have been expected, since, as already noted in the case of the sand mixtures, the accumulated sulfuric acid would

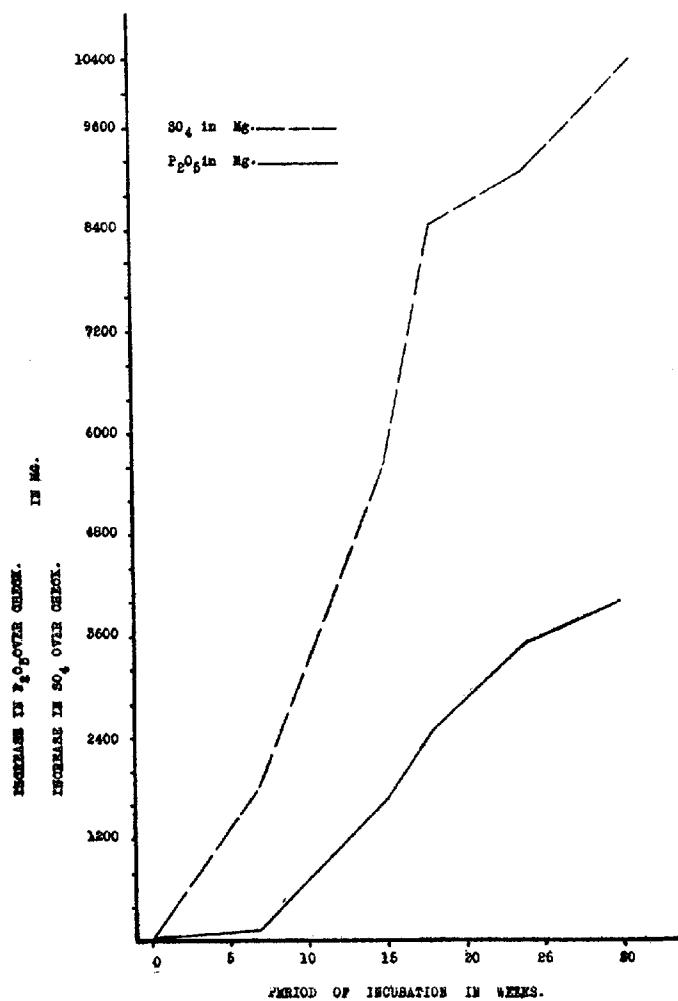


Fig. 3.—Curves showing the citrate-soluble  $P_2O_5$  and  $SO_4$  found in the composts made up with greenhouse soil during a period of 30 weeks.

tend to react with the tricalcic phosphate and to make it available according to the official method.

In the case of the Sassafras loam soil, we observe the gradual oxidation of a portion of the sulfur used in the sulfur-soil mixtures, the more intense oxidation of sulfur used in the sulfur-floats-soil mixtures, the accumulation of sulfates and also of citrate-soluble phosphates. It will be readily seen, at the same time, that the Sassafras loam was not as good a medium for the activities of the sulfur-oxidizing organisms as

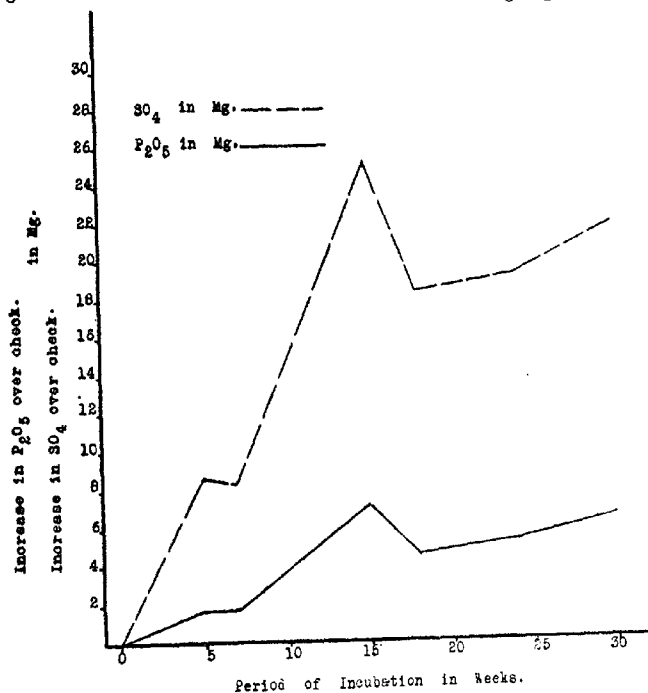


Fig. 4.—Curves showing the citrate-soluble  $P_2O_5$  and  $SO_4$  found in the composts made up with Sassafras loam soil during a period of 30 weeks.

was the greenhouse soil. In fact, it was inferior to the sand as a culture medium during the latter periods of the experiment, for, at the end of the thirtieth week, the accumulation of sulfates and of citrate-soluble phosphates was slighter in the sulfur-floats-Sassafras loam mixtures than it was in the corresponding sulfur-floats-sand mixtures. The inferiority of the Sassafras loam as a culture medium must be ascribed to its less favorable mechanical composition as well as to the possible accumulation of acid salts of iron and alumina that may have exerted a toxic effect on the microorganisms.

In concluding the consideration of the data contained in Table V, mention should be made of the ratio of oxidized sulfur to available phosphoric acid. It will be observed that, in the sulfur-floats-sand or sulfur-floats-soil mixtures, the quantities of  $P_2O_5$ , expressed in milligrams, closely correspond to the quantities of sulfur expressed in milligrams. Thus, in the sand mixtures there were found at the end of the fifteenth week 261.64 mg. of  $P_2O_5$  and 284.36 mg. of oxidized sulfur. In the corresponding greenhouse soil mixtures there were found at the end of the fifteenth week 1839.74 mg. of citrate-soluble  $P_2O_5$  and 1866.78 mg. of sulfur in the form of sulfates. In the corresponding Sassafras loam soil mixtures there were found at the end of the fifteenth week 713.03 mg. of phosphoric acid and 847.23 mg. of sulfur. It would appear, therefore, that, under the conditions of the experiment, the oxidation of one part of sulfur made available an equivalent of one part of  $P_2O_5$ . The significance of this relation is obvious when considered from the standpoint of the composting of floats with sulfur for the purpose of making the phosphorus in the former more readily available to growing vegetation.

*The Formation of Available Phosphoric Acid in Constant Quantities of Soil with a Varying Content of Sulfur and Floats*

In order that the phosphorus in floats may be made available most advantageously under farm conditions, suitable proportions of soil, moisture, sulfur, floats and organic matter must be employed. In so far as the sulfur and floats are concerned, concentrated composts would be, everything else being equal, more economical than less concentrated composts. If, let us say, 1000 pounds of floats could be treated effectively in a one-ton mixture, the cost of the treatment would be less than if the same quantity of floats were rendered available in a two or three-ton mixture. It was proposed, in outlining the experiment described below, to determine, if possible, the best proportions of sulfur to floats. Varying quantities of these ingredients were mixed with 100-gm. quantities of Sassafras loam soil. The mixtures were maintained at the moisture content of about 24 per cent. At the end of six weeks and of twelve weeks, respectively, the acidity and ammonium citrate and water-soluble phosphoric acid were determined. The arrangement of the experiment and the results secured are shown in Table VI.

It will be observed that 15 gm. of floats were mixed with sulfur varying in quantity from 1 gm. to 15 gm. and that 5 gm. of sulfur were mixed with floats varying in amount from 3 gm. to 30 gm. The results indicate that at the end of six weeks practically maximum amounts of available phosphoric acid were obtained from the 15 gm. of floats used with 1 gm. of sulfur. The actual maximum, both of available phosphoric acid and of acidity, was obtained when the 15 gm. of floats were used together with 9 gm. of sulfur. At the end of twelve weeks,

the maximum amount of available phosphoric acid was obtained from 15 gm. of floats when the latter were used together with 9 gm. of sulfur. On the other hand, the maximum degree of acidity was developed when 15 gm. of floats were used together with 7 gm. of sulfur.

With the quantity of sulfur kept constant, viz: 5 gm., and the quantity of floats variable, the maximum quantity of available phos-

TABLE VI  
INFLUENCE OF VARYING PROPORTIONS OF SULFUR AND FLOATS ON  
ACCUMULATION OF AVAILABLE PHOSPHORIC ACID

Lab. No and Additions	At End of Six Weeks						At End of Twelve Weeks					
	Acidity, cc. of N/50 KOH	Ammonium Citrate- Soluble $P_2O_5$			Water Sol- uble $P_2O_5$			Acidity, cc. of N/50 KOH	Ammonium Citrate- Soluble $P_2O_5$			Increase per gm. of Floats, mg.
		Mg.	Increase over Check, mg.	Increase per gm. of Floats, mg.	Mg.	Increase over Check, mg.	Mg.		Increase over Check, mg.			
1. None .....	7.50	99.14	.....	.....	13.20	.....	7.90	100.40	.....	.....	.....	.....
2. 15 gm. Floats....	6.50	155.75	.....	.....	18.75	.....	7.95	150.00	.....	.....	.....	.....
3. 15 gm. Floats, 1 gm. Sulfur	400.00	233.88	78.13	5.21	30.47	11.72	520.00	310.99	160.99	.....	10.73	.....
4. 15 gm. Floats, 2 gm. Sulfur	436.00	239.92	84.17	5.61	31.71	12.96	484.00	311.48	161.48	.....	10.77	.....
5. 15 gm. Floats, 3 gm. Sulfur	412.00	226.94	71.19	4.75	30.23	11.48	500.00	312.23	162.23	.....	10.81	.....
6. 15 gm. Floats, 5 gm. Sulfur	408.00	238.33	83.08	5.54	33.20	14.45	498.00	314.71	164.71	.....	10.98	.....
7. 15 gm. Floats, 7 gm. Sulfur	456.00	252.71	96.96	6.46	35.18	16.43	676.00	410.60	260.60	.....	17.37	.....
8. 15 gm. Floats, 9 gm. Sulfur	456.00	258.65	102.90	6.86	29.23	10.48	564.00	480.73	330.73	.....	22.05	.....
9. 15 gm. Floats, 11 gm. Sulfur	392.00	238.83	83.08	5.54	30.23	11.48	466.00	329.57	179.57	.....	11.97	.....
10. 15 gm. Floats, 15 gm. Sulfur	448.00	243.80	88.05	5.87	30.72	11.97	588.00	370.46	220.46	.....	14.69	.....
11. 3 gm. Floats, 5 gm. Sulfur	432.00	137.75	27.30	9.10	19.23	4.92	552.00	142.98	32.66	.....	10.89	.....
12. 5 gm. Floats, 5 gm. Sulfur	404.00	176.39	58.45	11.69	19.82	4.77	658.00	340.73	223.80	.....	44.76	.....
13. 8 gm. Floats, 5 gm. Sulfur	360.00	189.28	59.98	7.49	22.55	5.39	496.00	236.65	109.80	.....	13.72	.....
14. 12 gm. Floats, 5 gm. Sulfur	392.00	196.22	51.84	4.32	28.00	10.36	508.00	276.30	136.23	.....	11.35	.....
15. 20 gm. Floats, 5 gm. Sulfur	444.00	284.43	109.89	5.49	37.30	16.70	550.00	365.51	198.99	.....	9.95	.....
16. 30 gm. Floats, 5 gm. sulfur	496.00	346.85	134.61	4.49	34.93	10.63	520.00	432.41	232.83	.....	7.76	.....

phoric acid was secured from the largest amount of floats. This was true of both the six and twelve week periods. It would seem, all told, that, under favorable conditions, one part of sulfur and two parts of floats may constitute a satisfactory combination for the production of available phosphoric acid. This conclusion is at present tentative, in view of the somewhat conflicting data and the short period of the experiment. It is



strengthened, however, by the results already recorded in Tables I to IV. It should be added here that, sulfur being the most expensive of the constituents, it would be desirable to use as wide a ratio of floats to sulfur as may be consistent with the greatest yield of available phosphoric acid.

#### *Moisture as a Factor in Sulfur Oxidation*

The importance of the moisture factor in the oxidation of sulfur by microorganisms became evident during the progress of the experiments described in the foregoing pages. For this reason it seemed desirable to make a more careful study of the influence of varying amounts of moisture on the oxidation of sulfur. One hundred-gm. portions of the Sassafras loam soil were used and the mixtures were made up in the same manner as those already described. "Optimum Moisture" as used in this experiment is 50 per cent of the water-holding capacity of the soil mixtures. The optimum moisture for the soil-floats mixtures was 24.25 c.c. and for the soil-floats-sulfur mixtures 24.00 c.c. After incubation for 6 weeks the mixtures were examined for acidity and for ammonium citrate and water-soluble phosphoric acid. The plan of the experiment and the results obtained are recorded in Table VII, and are shown graphically in figure 5.

The results presented in Table VII show that the moisture content has an important influence on the processes in which sulfur undergoing oxidation, renders the phosphoric acid of the floats available. However, where there was no sulfur to be oxidized, as was the case where floats only were used, no distinctive differences are noted. These statements hold true for both citrate and water-soluble phosphorus. Another fact which is clearly brought out for the soil-floats-sulfur mixtures is that a low and a high moisture content retards the production of citrate and water-soluble phosphorus.

A more detailed study of the results obtained with the soil-floats mixtures show that portions 5 and 6 which received  $1\frac{1}{4}$  and  $1\frac{1}{2}$  optimum moisture, respectively, contained slightly less available phosphoric acid than the others. This is due either to more thorough fixation or to the reversion of the soluble phosphoric acid originally present in the floats. It is possible, however, that the large proportion of moisture affected somewhat the ammonium citrate determinations.

With the soil-floats-sulfur mixtures the acidity increased from 36 c.c., expressed as N/50 KOH where  $\frac{1}{4}$  optimum moisture was added, to 484 c.c. with an addition of moisture equivalent to  $\frac{3}{4}$  optimum. A further addition of moisture slightly decreased the acidity, but when  $1\frac{1}{2}$  optimum moisture was added there was a large decrease. The acidity in this case was equivalent to 60 c.c. N/50 KOH.

A comparison of the amounts of citrate-soluble phosphoric acid in the soil-floats-sulfur mixtures will show that the increase was from 165.26

mg. in the composts receiving  $\frac{1}{4}$  optimum, to 289.21 mg. of  $P_2O_5$  in the portions receiving  $\frac{1}{4}$  optimum moisture. It seems, therefore, that  $\frac{1}{4}$  optimum moisture conditions are the best in this soil for maximum results, in rendering available insoluble phosphates such as raw rock phosphate.

The results apparently show that the maximum oxidation of sulfur takes place under  $\frac{3}{4}$  optimum moisture conditions, as is shown by the acidity, but the conditions for the greatest solvent effect on the rock phosphate are found in the soils with a high moisture content. The reversion of some of the phosphorus which had been made available may be offered as a possible explanation of the relations just noted.

TABLE VII  
THE INFLUENCE OF MOISTURE ON THE ACCUMULATION OF ACIDITY AND OF AVAILABLE PHOSPHORIC ACID

Lab. No. and Additions	Moisture Proportion of Optimum	$H_2O$ , cc	At End of Six Weeks				At End of Twelve Weeks			
			Acidity, cc. of N/50 KOH	Citrate-Soluble $P_2O_5$ in Composts		Water-Soluble $P_2O_5$ in Composts, mg.	Acidity, cc. of N/50 KOH	Citrate-Soluble $P_2O_5$ in Composts		Water-Soluble $P_2O_5$ in Composts, mg.
				$P_2O_5$ , mg.	Increase over Check, mg.			$P_2O_5$ , mg.	Increase over Check, mg.	
1. 15 gm. Floats.....	$\frac{1}{4}$	6.10	10.00	172.77	.....	13.09	9.80	170.00	.....	12.60
2. 15 gm. Floats.....	$\frac{1}{2}$	12.15	14.00	176.53	.....	13.63	9.30	170.80	.....	12.60
3. 15 gm. Floats.....	$\frac{3}{4}$	18.20	7.00	170.27	.....	16.60	10.00	172.80	.....	14.40
4. 15 gm. Floats.....	1	24.25	14.00	172.77	.....	14.12	9.50	168.10	.....	12.80
5. 15 gm. Floats.....	$1\frac{1}{4}$	30.35	11.00	169.02	.....	15.86	10.10	170.00	.....	12.40
6. 15 gm. Floats.....	$1\frac{1}{2}$	36.40	20.00	167.76	.....	17.59	9.60	167.90	.....	13.68
7. 15 gm. Floats, 5 gm. Sulfur...	$\frac{1}{4}$	6.00	36.00	165.26	-7.51	15.86	200.00	230.10	60.10	25.90
8. 15 gm. Floats, 5 gm. Sulfur...	$\frac{1}{2}$	12.00	32.00	179.03	2.50	16.85	278.40	320.00	149.20	46.70
9. 15 gm. Floats, 5 gm. Sulfur...	$\frac{3}{4}$	18.00	484.00	256.03	85.76	27.65	495.20	500.00	317.20	118.20
10. 15 gm. Floats, 5 gm. Sulfur...	1	24.00	448.00	282.95	110.18	31.22	580.40	580.40	412.30	126.00
11. 15 gm. Floats, 5 gm. Sulfur...	$1\frac{1}{4}$	30.00	440.00	289.21	120.19	31.22	651.00	620.30	450.30	131.40
12. 15 gm. Floats, 5 gm. Sulfur...	$1\frac{1}{2}$	36.00	60.00	190.30	22.54	14.87	400.10	390.00	222.10	49.80

The amount of water-soluble phosphoric acid in the composts is not large, but the results show that optimum and  $\frac{1}{4}$  optimum moisture conditions give the best results.

The results secured at the end of 12 weeks confirm, in most respects, those secured at the end of the shorter period. It will be observed, however, that the maximum accumulation of acid occurred here with  $\frac{1}{4}$  optimum moisture content. The maximum accumulation of citrate and water-soluble phosphoric acid occurred with the same moisture content. When the moisture content was increased beyond  $\frac{1}{4}$  optimum the accumulation of acidity and of available phosphoric acid was depressed.

*Biological Factors*

The results already recorded naturally raise the question as to the nature of the biological factors involved in the oxidation of sulfur to sulfuric acid. Other investigators have apparently demonstrated that the oxidation of sulfur in soils is a biological process. It seems desirable, nevertheless, to determine in the present experiment the extent to which biological factors had to do with the oxidation of sulfur. To this end, 100-gm. portions of Sassafras loam soil were arranged in three

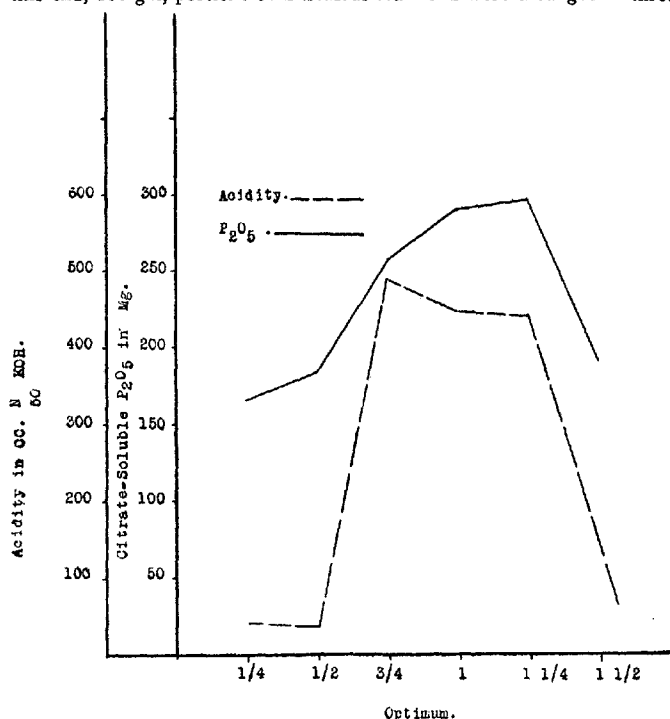


Fig. 5.—Curves showing the influence of moisture content on the availability of phosphorous and acidity: length of incubation period 6 weeks.

groups, designated, respectively, as (1) Untreated (2) Sterilized and Inoculated (3) Unsterilized and Inoculated. The inoculation was effected by means of a soil infusion derived from soil supposedly well provided with sulfur-oxidizing organisms. In making the inoculation, 10-gm. portions of soil were shaken with 100 c.c. of water, and 5 c.c. of the infusion thus prepared were added to each 100-gm portion of the Sassafras loam soil. The soil to be sterilized was autoclaved at 15 pounds for 15 minutes. The arrangement of the series in question, together

with the results secured at the end of 7 and 14 weeks, respectively, are shown in Table VIII.

In drawing any conclusions from the results reported above, it appears necessary to accredit the soil infusion with bringing about an increase in the amount of available phosphorus. Thus, the sulfur-floats-soil mixture, which contained unsterilized and inoculated soil, contained, at the end of 7 weeks, 434.44 mg. of phosphoric acid, and, at the end of 14 weeks, 634.37 mg. of phosphoric acid as against corresponding quantities of 250.42 mg. of phosphoric acid and 339.48 mg. of phosphoric acid, respectively, for the corresponding portions which were unsterilized but not inoculated. Other experiments, both with crude and pure cultures of soil microorganisms, are now in progress for the purpose of securing additional information on this point.

TABLE VIII  
INFLUENCE OF BIOLOGICAL FACTORS ON THE AVAILABILITY OF THE  
PHOSPHORIC ACID IN FLOATS

Additions	Sterilized and Inoculated		Unsterilized and Inoculated		Untreated	
	Acidity c.c.	Citrate-Soluble $P_2O_5$ mg.	Acidity c.c.	Citrate-Soluble $P_2O_5$ mg.	Acidity c.c.	Citrate-Soluble $P_2O_5$ mg.
AT END OF 7 WEEKS						
None .....	20.00	65.10	10.00	73.37	10.50	70.86
5 gm. Sulfur .....	188.00	83.88	818.00	97.66	402.00	90.14
15 gm. Floats .....	31.50	121.44	10.00	159.00	10.75	164.01
5 gm. Sulfur, 15 gm. Floats	535.00	391.88	562.00	434.44	484.00	260.42
AT END OF 14 WEEKS						
None .....	16.00	68.30	12.10	70.40	11.05	71.00
5 gm. Sulfur .....	408.00	78.90	1080.00	82.10	648.00	87.50
15 gm. Floats .....	16.50	123.90	12.10	140.10	11.10	139.60
5 gm. Sulfur, 15 gm. Floats	496.00	491.88	612.00	634.37	458.00	339.49

#### SUMMARY

The theory recently advanced by the senior author that the oxidation of sulfur by microorganisms is of value in making available the phosphorus of mineral phosphates has been substantiated by the following results (subject to the limitations of the experiments).

1. One hundred-gm. portions of sea sand, Sassafras loam and a greenhouse soil to which had been added manure, were composted with sulfur and floats and analysed periodically for acidity and available phosphoric acid by the official method. In the sand cultures the acidity of the sulfur-floats mixture increased with the period of incubation until at the end of the eighteenth week it was four times as great as where sulfur

had been applied alone. That the sulfonation process gained in intensity most markedly after the eighteenth week is supported by the data showing that the available phosphoric acid in the sulfur-floats mixture increased markedly compared with the sulfur treatment from the eighteenth to thirtieth week.

2. The greenhouse soil, being rich in nitrogen and mineral plant-food as well as organic matter proved superior to sand as a culture medium. The increase in acidity was much greater than in sand. The sulfur alone yielded a maximum accumulation larger than that of the sulfur-floats mixture. But since 85 per cent of the phosphoric acid present in the latter was made available it is evident that the acid which resulted from the oxidation of the sulfur in the sulfur-floats mixture had reacted with the tricalcic phosphate. The results obtained with the Sassafras loam differed in degree rather than in kind from those already recorded for the sand and greenhouse soils, showing that the biological changes throughout the experiment were limited in a definite way by the physical and chemical properties of the medium.

3. Analyses for the accumulation of water-soluble phosphoric acid showed increases in all of the mixtures which had received additions of both sulfur and floats.

4. In both soils and sand, the oxidation of sulfur as measured in terms of sulfates, paralleled the increase of available phosphoric acid throughout the incubation period of 30 weeks. The tricalcium phosphate hastened the oxidation of sulfur by furnishing phosphorus for the life activities of the sulfur organisms and by neutralizing the free acid which being formed would tend to inhibit the proper development of the sulfur-oxidizing organisms.

5. Investigation of moisture as a factor in sulfur oxidation revealed the fact that high or low moisture contents retarded the production of acidity, as well as of the citrate and water-soluble phosphoric acid. One and one-fourth optimum moisture conditions appeared to be best in sassafras soil for maximum results in rendering available raw rock phosphate. It was shown also that in a comparison of (1) untreated, (2) sterilized and inoculated, (3) unsterilized and inoculated soils, the biological factor proved influential in the oxidation of sulfur and the making available of raw rock phosphate.

6. Data are advanced which indicate that in composting under farm conditions, one part of sulfur and two parts of floats may constitute a satisfactory combination for the production of available phosphoric acid. While several points are still under investigation, there is good reason to believe that in field practice, inert phosphates may best be made available by sulfur oxidation in a relatively concentrated medium *e. g.* in a compost heap containing a relatively large proportion of both phosphates and sulfur.

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# THE ORGANIC MATTER OF THE SOIL: II. A STUDY OF CARBON AND NITROGEN IN SEVENTEEN SUCCESSIVE EXTRACTS; WITH SOME OBSERVATIONS ON THE NATURE OF THE BLACK PIGMENT OF THE SOIL<sup>1</sup>

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## INTRODUCTION

It has already been shown in the first paper of this series (2) that:

1. Humus, the "matière noire" of Grandeau (3), does not consist entirely of a black compound or compounds but contains a large proportion of almost colorless substances, the presence of which is normally masked by the black color.
2. Extracts can be obtained from unchanged vegetable materials which are very similar in solubility, color, etc., to "humus" extracts prepared from soils.
3. A 4 per cent solution of sodium hydroxide does not extract either the same substances or the same quantity of substances as does a 4 per cent solution of ammonium hydroxide.
4. All of the mineral soils tested contain a "soil pigment" which is soluble in ammonia and very dilute sodium hydroxide to form intensely black solutions, but which is insoluble in sodium hydroxide of 4 per cent concentration.
5. This soil pigment contains a relatively small proportion of the soil nitrogen and it was suggested that it probably had but little importance, *per se*, in the problem of soil fertility.

In view of the above findings it seemed advisable to attempt to isolate this soil pigment in a pure form. Such an attempt is recorded in this paper. It is to be regretted that the final preparations contain such a high percentage of ash as probably to invalidate the ultimate analyses, but it is impossible to carry the investigation further at the present time, and the results to date seem worthy of record.

<sup>1</sup>The work reported in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soils Chemist.

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## EXPERIMENTAL

The soil selected for this investigation was from a bulk sample of the first 6 inches of the Fargo silt loam type (4), the air-dry soil containing 12.70 per cent of moisture. The soil contained 9.90 per cent of carbonate  $\text{CO}_2$ , 10.04 per cent of organic carbon and 0.908 per cent of nitrogen, all calculated on the basis of oven-dry soil.

In the above mentioned paper (2) the writer has shown that while the soil pigment is insoluble in 4 per cent sodium hydroxide, it is readily soluble in the dilute alkaline solution formed when distilled water is added to the soil residue remaining after siphoning off the 4 per cent sodium hydroxide solution. An attempt was made to utilize this phenomenon for the purpose of separating the soil pigment. One kg. of the air-dry soil, ground to pass a 1-mm. sieve, was leached in 50 gm. lots on filters with 1 per cent HCl until the filtrate gave no test for calcium, and the excess of acid removed by washing with water. The leached soil was then removed to 2½-liter bottles, the soil residue from 100 gm. of the original air-dry soil being placed in each bottle and 2 liters of 4 per cent NaOH added to each. The bottles were shaken several times each day for 3 days and then the soil was allowed to settle until the supernatant liquid appeared to be free from clay, 3 to 4 days usually being allowed. The supernatant liquid was siphoned off as completely as possible without disturbing the soil, measured and united in a large container, 16,900 c.c. being removed. This constituted "NaOH Solution A." Fresh 4 per cent NaOH solution was added to the soil residue until 2 liters of NaOH were again contained in each bottle and the process repeated until there had been 9 successive extractions with 4 per cent NaOH.

Carbon and nitrogen determinations were made of each solution, the carbon being estimated by wet combustion in the apparatus figured in the preceding paper (2), corrections being made for the  $\text{CO}_2$  of carbonates in the sodium hydroxide solutions.<sup>1</sup>

The color of the solutions rapidly decreased in intensity, the last two extractions being practically colorless.

Following the nine extractions with sodium hydroxide the contents of one of the bottles was made slightly acid with sulfuric acid, the precipitated soil washed by decantation until practically free from sulfates, then poured upon a filter and washed free of sulfates, removed from the filter, dried, weighed and powdered to pass a 0.5-mm. sieve. From the

<sup>1</sup>It was impossible to secure accurate organic carbons on the large volume (45,350 c.c.) of HCl extract and washings, so that the organic carbon in this portion was determined by difference.

Ten gm. of the air-dry soil were leached on an alundum filter with 1 per cent HCl until free from calcium, and washed free of chlorides with distilled water. The soil was then washed from the filter into an evaporator, dried and weighed. The 10 gm. of original air-dry soil, containing 0.8764 gm. of organic carbon, gave 6.4452 gm. of dry leached soil containing (1) 13.12 per cent, and (2) 13.10 per cent, or an average of 13.11 per cent, equivalent to 0.8450 gm. of organic carbon. The HCl leachings therefore dissolved 0.0314 gm. of organic carbon.

original 87.30 gm. of soil (dry) there was obtained a residue weighing 41.5 gm. or 47.53 per cent of the original soil weight. This residual soil contained 6.27 per cent of organic carbon and 0.346 per cent of nitrogen.

A "humus" determination was made on this residual soil by shaking 5 gm. of the sample with 750 c.c. of 4 per cent  $\text{NH}_4\text{OH}$  for one week, flocculating the clay with ammonium carbonate, filtering and evaporating 250-c.c. portions to dryness in tared silica dishes. Duplicate determinations gave 8.38 and 8.45 per cent of the residual soil as ash-free humus of an average of 8.42 per cent. The humus-ash percentages were 1.76 and 1.80 per cent.

The "humus" solution was jet black and on drying the product scaled away from the dish in flakes where the deposit had any appreciable thickness. The dissolved material apparently consisted of the black soil pigment combined with ammonia, inasmuch as the product after drying at  $105^\circ \text{C}$ . for 12 hours was still readily soluble in water. Detmer (1) has observed that a soluble "ammonium humate" can be formed by dissolving "humic acid" in ammonia and evaporating the mixture to dryness. The properties of his "salt" resemble very closely those of the writer's product.

To the remaining nine soil residues, distilled water was added to 2 liters volume, the mixture thoroughly shaken and 5 gm. of crystallized sodium sulfate added to each bottle to flocculate the clay. After several shakings the soil was allowed to settle and the black supernatant liquid siphoned off, this constituting Pigment Solution 1. Additional 0.25 per cent sodium sulfate solution was added to the residue and the process repeated until six pigment solutions had been removed.<sup>1</sup>

Pigment Preparation I was obtained from the combined Pigment Solutions 1, 2 and 3, and the remaining solutions 4, 5 and 6 furnished Pigment Preparation II (see below).

The residual soil from the pigment extractions was acidified slightly with sulfuric acid, washed by decantation to the absence of acid reaction, poured upon filters and washed to the absence of sulfates, dried, weighed and powdered to pass a 0.5-mm. sieve. There was thus obtained a light gray residue weighing 330.5 gm. as compared with an original weight of 785.7 gm. (900 gm. air-dry) and containing 3.59 per cent of organic carbon and 0.211 per cent of nitrogen.

Inasmuch as it was thought probable that there was still some of the "soil pigment" remaining in the residue, "humus" was determined on the resulting residue. Two 15-gm. portions were leached with 1 per cent  $\text{HCl}$  and washed free of chlorides as in the usual humus method. This treatment was resorted to in order to get the soil in as near as possible the same physical state as it would have been had it not been previously dried and ground. The 15 gm. of soil were then added to 750-c.c. portions

<sup>1</sup> Fifteen c.c. of 20 per cent  $\text{NaOH}$  were added to each 2000 c.c. of solution used for the extraction of Pigment Solutions 5 and 6.

of 4 per cent  $\text{NH}_4\text{OH}$  in separate bottles and shaken for one week in a shaking machine so arranged that the bottles were rotated sidewise "wheel fashion" so that the soil was continually falling through the solution. At the end of a week the bottles were removed, the clay precipitated in a portion of the solution with  $\text{K}_2\text{SO}_4$  and carbon determined on the jet black solution as in the writer's earlier directions (2). The remaining portion was used for "humus" determinations after flocculating the clay with ammonium carbonate as in Rather's (5) method. The residual soil was found to contain 1.54 per cent of humus (ash-free), 0.74 per cent of "humus ash," and 0.95 per cent of "humus carbon" indicating 62.25 per cent of carbon in the humus. This carbon percentage agrees fairly well with the carbon content of Pigment Preparation I, i. e., 61.33 per cent (see later).

The summarized analytical data are shown in Tables I and II. Figure 1 is a graphic representation of the data of Table II.

The analytical results obtained on this series are conclusive proof of the accuracy of my wet combustion apparatus for the determination of organic carbon. The increase of 1.534 gm. of carbon amounts to an error of 1.76 per cent of the total carbon. Inasmuch as carbon was determined on 50-c.c. portions of the solution, there would have been enough solution to make 5,012 carbon determinations, not considering the 45,350 c.c. of the HCl extract; a gain of 1.534 gm. of carbon, therefore, amounts to an average gain of only 0.0003 gm. of carbon per determination. A nitrogen gain of 0.1590 gm. N is similarly an average gain of only 0.00013 gm. N per determination, not counting errors in the nitrogen content of the original soil nor of the final soil residue.

*Pigment Preparation 1.* Pigment Solutions 1, 2 and 3 were united and acidified with sulfuric acid. A jet black flocculent precipitate separated leaving the supernatant liquid absolutely colorless. The precipitate was washed by decantation with distilled water until the supernatant liquid began to take on a brown tint showing that the pigment was beginning to form a colloidal solution. Sufficient sodium hydroxide solution was then added to form a 0.2 per cent solution. The precipitated pigment was dissolved to a jet black solution, which was only faintly alkaline to litmus. This solution was poured upon a large filter and the filtrate diluted to 7 liters in volume. One liter of sodium hydroxide solution containing 320 gm. of sodium hydroxide was then added, thus making the solution to contain 4 per cent of sodium hydroxide. The black pigment was precipitated in this concentration of alkali, leaving a supernatant liquid which appeared very dark when in large bulk but which was relatively light colored when in small volume. This supernatant liquid was analyzed for organic carbon and nitrogen and gave a ratio of C/N of 15.77. This is decidedly lower than the C/N ratio of the original solution, 21.0, and would indicate that the substances dissolved here were perhaps normally soluble in 4 per cent NaOH. It is

to be noted that this ratio of 15.77 logically follows the C/N ratio of NaOH solution 1.

The supernatant liquid was removed from the pigment as completely as possible by centrifuging, and the solid mass of pigment added to 8 liters of distilled water in which it readily dissolved to form a jet black solution. This solution was filtered by gravity through double filters and

TABLE I  
SUMMARIZED ANALYTICAL DATA ON THE SOILS AND SOLUTIONS<sup>1</sup>

	Volume of Solution Removed from 1 kg. Soil <sup>2</sup>	Organic Carbon			Nitrogen		
		Per cent I	Per cent II	Per cent Av.	Per cent I	Per cent II	Per cent Av.
1% HCl Extract .....	c.c.						
NaOH Solution—	45,350	0.353	0.368	0.360	0.025	0.023	0.024
A.....	16,900	3.353	3.323	3.338	0.363	0.364	0.363
B.....	17,100	1.126	1.145	1.136	0.138	0.138	0.138
C.....	17,300	0.640	0.626	0.633	0.080	0.080	0.080
D.....	17,600	0.442	0.424	0.433	0.053	0.053	0.053
E.....	17,700	0.298	0.299	0.298	0.033	0.033	0.033
F.....	17,300	0.252	0.252	0.252	0.026	0.027	0.026
G.....	17,500	0.173	0.180	0.177	0.018	0.017	0.018
H.....	17,000	0.143	0.151	0.147	0.012	0.012	0.012
I.....	15,900	0.101	0.108	0.104	0.010	0.009	0.009
Residual Soil from NaOH Solution I..	.....	3.007	2.953	*2.980	0.163	0.165	*0.164
Pigment Solution—							
1.....	15,890	0.168	0.160	0.164	0.008	0.009	0.009
2.....	18,890	0.475	0.474	0.475	0.020	0.021	0.020
3.....	17,112	0.595	0.591	0.593	0.029	0.029	0.029
4.....	14,445	0.292	0.290	0.291	0.015	0.015	0.015
5.....	14,110	0.164	0.183	0.173	0.006	0.006	0.006
6.....	15,890	0.104	0.102	0.103	0.006	0.003	0.004
Residual Soil from Pigment Solution 6	.....	1.521	1.503	1.512	0.088	0.089	0.088
Total .....	.....	.....	.....	10.189	.....	.....	0.927
Original Soil .....	.....	10.076	10.020	10.040	0.904	0.913	0.908

<sup>1</sup> All percentages are based on the weight of the original soil (dry at 105°).

<sup>2</sup> The volume for the pigment solutions has been increased 1/9 to correspond to 1 kg. of soil instead of 900 gm. actually extracted (see text).

\* Not included in the additions for "Total."

the filtrate acidified slightly with sulfuric acid. The precipitate was washed by decantation until it began to form a colloidal solution, then poured on a filter and washed until practically free from sulfates. The large mass of gelatinous precipitate, probably approximating 800 c.c. in bulk, was dried on a water bath. In drying it cracked into small pieces which resembled anthracite in color and hardness when perfectly dry. The yield was approximately 16 gm.

The crude pigment was powdered, extracted on an alundum filter with hot water to remove traces of sulfates, then extracted in a Soxhlet apparatus with alcohol. The extract rapidly darkened and finally became deep orange in color. On evaporating off some of the alcohol and cooling, a very considerable quantity of colorless crystals separated. These crystals have not as yet been identified.

The extracted pigment was dried at 105° C. and analyzed for carbon, hydrogen, nitrogen and ash in the usual manner. The product as prepared forms a jet black amorphous powder, insoluble in alcohol, ether, chloroform or any of the usual organic solvents, insoluble in dilute acids,

TABLE II  
SHOWING THE PERCENTAGE OF THE TOTAL CARBON AND NITROGEN IN THE  
DIFFERENT SOILS AND SOIL EXTRACTS

	Gm. C.	Per cent Total C.	Gm. N.	Per cent Total N.	C/N Ratio
1000 gm. Original Soil .....	87.640	.....	7.9300	.....	11.05
1% HCl Extract .....	3.137	3.58	0.2192	2.76	14.32
NaOH Solution—					
A.....	29.139	33.25	3.1730	40.91	9.18
B.....	9.910	11.31	1.2034	15.17	8.24
C.....	5.524	6.30	0.6972	8.79	7.92
D.....	3.782	4.32	0.4611	5.81	8.20
E.....	2.607	2.98	0.2867	3.61	9.09
F.....	2.199	2.51	0.2318	2.92	9.49
G.....	1.756	2.00	0.1505	1.90	11.67
H.....	1.278	1.46	0.1054	1.33	12.13
I.....	0.919	1.05	0.0668	0.84	13.76
Residual Soil from NaOH Solu. I	126.020	129.69	11.4350	118.09	18.12
Pigment Solution—					
1.....	1.421	1.62	0.0747	0.94	19.03
2.....	4.152	4.74	0.1813	2.29	22.90
3.....	5.181	5.91	0.2498	3.15	21.06
4.....	2.537	2.89	0.1271	1.60	19.96
5.....	1.531	1.75	0.0522	0.66	29.34
6.....	0.901	1.03	0.0365	0.29	24.66
Residual Soil from Pig't Solu. 6	13.200	15.06	0.7730	9.75	17.08
Total .....	89.174	101.76	8.0900	101.82	.....

<sup>1</sup> Not included in the additions for "Total."

soluble in concentrated sulfuric acid to a black solution, dissolving slightly in hot concentrated nitric acid to a red-brown color, soluble in ammonia or very dilute solutions of the fixed alkalis to intensely black solutions, from which the pigment can be again precipitated by solutions of the heavy metals or by acidification.

When alkaline solutions of the pigment are dialyzed in collodion bags, the excess of alkali escapes but no pigment passes through. In this manner neutral solutions of the pigment can be easily prepared, which yield a black water-soluble residue when evaporated to dryness. These probably correspond in nature to the ammonium compound obtained by evaporating an ammoniacal solution of the pigment. Whether we are

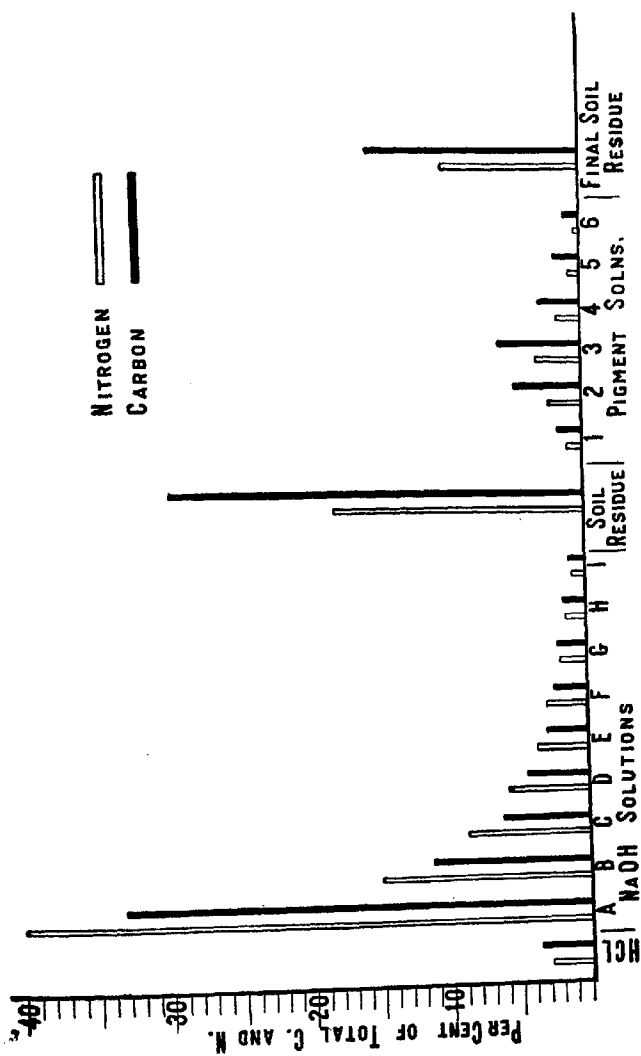


Fig. 1.—A graphic representation of the data in Table II.



dealing with a true salt in this instance can be decided only by further experimentation on purer material. The following analytical data were obtained on the sample:

Ash determinations indicated 37.50, 37.74 and 37.16 per cent or an average of 37.47 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 61.28, 61.59 and 61.12 per cent C, or an average of 61.33 per cent of carbon calculated to an ash-free basis.

Hydrogen determinations gave 4.35, 4.25 and 4.37 per cent hydrogen, or an average of 4.36 per cent calculated ash-free.

Nitrogen determinations gave 2.79 and 2.82 per cent N, or an average of 2.81 per cent calculated ash-free.

Sulfur was not tested for because of the high ash content and also because sulfuric acid had been used to precipitate the pigment.

These analyses therefore would indicate an approximate content of:

Carbon .....	61.3 per cent
Hydrogen .....	4.3 per cent
Nitrogen .....	2.8 per cent
Oxygen .....	31.6 per cent

It is useless to compare these analyses with the analyses of "humic acids," etc. in the literature until this product can be prepared in a purer state. It is of interest to note, however, that the analyses approximate those of Detmer's (1) "humic acid," which contained 60.74 per cent C, 4.48 per cent H, and 35.78 per cent O. Detmer's product contained smaller amounts of nitrogen, ranging from 0.79 per cent to 0.18 per cent, and he was inclined to the belief that his "humic acid" was nitrogen-free. The physical properties of his material are almost identical with those of the soil pigment used by the writer. He does not state, however, whether or not his "humic acid" is insoluble in 4 per cent NaOH and soluble in the very dilute solutions; this would appear to be the distinguishing feature of the soil pigment.

The high ash content consists presumably of colloidal clay which was not removed by the preliminary treatment. This clay undoubtedly holds a certain amount of combined moisture that was not driven off by the drying at 105° C. so that it appears probable that the hydrogen content as determined is too high. Some diminution of ash could be secured by dissolving the pigment in ammonia and precipitating the clay with ammonium carbonate. This was what was actually done in the humus determination on the residual soil from NaOH solution 1. This soil residue was found to contain 8.42 per cent of humus with 1.78 per cent of humus ash, or an ash content of only 17.45 per cent in the materials not precipitated by the ammonium carbonate. The ammonia treatment would be far more rapid than the sodium hydroxide process but was not

utilized because of the danger of introducing ammonia into the compound and thus increasing the nitrogen content. Inasmuch as the writer's analyses now show the approximate nitrogen content, it is extremely desirable that some one should attempt to isolate the pigment by the ammonia process. Grandeau (3) found that when an ammoniacal solution of humus containing 53 per cent of ash was dialyzed against distilled water for 36 hours, the resulting residue in the dialyzer had an ash content of only 8 per cent. Perhaps this method could be employed in freeing the soil pigment from ash.

*Pigment Preparation 2.* This sample of pigment, weighing approximately 7 gm. was prepared from Pigment Solutions 4, 5 and 6, following much the procedure as was used for Pigment Preparation 1, with the exceptions that the solution of the pigment in dilute alkali was not precipitated by adding NaOH up to 4 per cent concentration and that hydrochloric acid was used for acidification instead of sulfuric. The final extractions were the same for both preparations. The following analytical data were obtained on the sample:

Ash determinations indicated 50.77 and 51.57 per cent, or an average of 51.17 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 55.82 and 56.23 per cent C, or 56.03 per cent of organic carbon calculated to an ash-free basis.

Hydrogen determinations gave 5.89 and 6.36 per cent, or 6.13 per cent calculated ash-free.

Nitrogen determinations gave 2.94 and 3.02 per cent N, or an average of 2.98 per cent calculated to an ash-free basis.

These analyses would indicate an approximate content of

Carbon .....	56.03 per cent
Hydrogen .....	6.13 per cent
Nitrogen .....	2.98 per cent
Oxygen .....	34.86 per cent

It will be noted that this preparation contains appreciably more hydrogen and less carbon than Preparation 1. This is exactly what would be expected from a sample having a higher ash content, if the ash consisted of clay. Apparently the clay has retained enough water of constitution to vitiate entirely the hydrogen analysis and to lower the carbon percentage appreciably.

#### SUMMARY

A sample of soil of the Fargo silt loam type was leached with 1 per cent hydrochloric acid and then extracted 9 consecutive times with fresh portions of a 4 per cent sodium hydroxide solution. A sample of the residual soil was removed at this point and analyzed, while the remain-

ing portions were further extracted for 6 consecutive times with very dilute (approximately 0.15 per cent concentration) sodium hydroxide solution. Following these extractions the remaining soil residue was dried and analyzed. The following conclusions are evident from the analytical data:

1. The writer's earlier observation that 4 per cent sodium hydroxide solution does not dissolve the black soil pigment is confirmed.

2. The soil pigment is soluble in very dilute sodium hydroxide solutions but precipitates from solution on the addition of sodium hydroxide in sufficient amount to make a 4 per cent solution.

3. The soil pigment is also precipitated from solution by salts of the heavy metals and by acidification; is not dialyzable and forms a stable water-soluble compound with ammonia when an ammoniacal solution is evaporated to dryness.

4. Two attempts were made to prepare the soil pigment in pure form but the resulting products contained such a high content of ash (37.47 per cent and 51.17 per cent, respectively) as probably to render the ultimate analysis unreliable. The analysis of the product with the least ash content gave C., 61.3 per cent; H., 4.3 per cent; N., 2.8 per cent; O., 31.6 per cent; all calculated to an ash-free basis.

5. The first six extractions with NaOH removed relatively more nitrogen than carbon from the soil, but the remaining three NaOH extractions as well as the six pigment solutions contained relatively more carbon than nitrogen. The final soil residue had a ratio of C/N very much higher than that of the original soil.

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## FERRIFICATION IN SOILS<sup>1</sup>

By

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### INTRODUCTORY

Iron has long been known as an essential plant-food constituent. The experiences of the early plant physiologists indicated that the formation of chlorophyll was dependent upon the presence of the element iron. It was recognized, of course, that other constituents were likewise necessary, but in the absence of iron plants soon took on a yellowish, or blanched appearance known as chlorosis, and died. Analyses failed to show the presence of iron in chlorophyll and hence its importance was concluded to be due to a stimulative catalytic, or some other unknown action.

Molisch (8), Raulin (11) and Benecke (1) believed that iron was necessary for the formation of protoplasm and that the chlorosis brought about by its absence was a secondary effect.

Many experimentalists have confirmed the work of the early investigators in showing that iron is essential for the proper development of plants, but little light has been thrown on the question of its exact function in the plant cell. Experiments along such lines would certainly prove extremely interesting and valuable.

While, therefore, the importance of iron to plants has long been recognized, it has always been believed that there was sufficient present in the proper condition in all soils to keep plants supplied for an indefinite period. Certain experiments have shown, however, that applications of iron salts to some soils may prove beneficial. Griffith (4) secured large increases in beans when ferrous sulfate was applied to the soil. Increases were likewise secured when the same salt was applied to turnips, meadow hay, mangel-wurzels, potatoes, wheat, tobacco, onions and cabbage. While there is some question regarding the exact action and cause of the value of the ferrous sulfate used in these experiments, Griffith reports the soils used as extremely low in iron content and the assumption is that the addition of iron benefited the crops. Soils, in various localities, have been found deficient in the element iron, and plants grown on them show this deficiency by their typical chlorotic appearance. It seems therefore, that iron fertilization of some soils may be rather important. On the other hand, Halligan (6) believes that enough iron is soluble in the weak soil acids to supply the needs of plants so that this material

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need not be considered when applying fertilizers. Russell and Hutchinson (13) assume likewise that iron salts are unnecessary as manures. Hall (5) is even more emphatic, in declaring the use of iron sulfate either for farm or garden crops as unnecessary. He insists that no direct evidence has yet been adduced for a beneficial effect of iron salts either on color or yield, and that experiments have never been conducted in a manner to raise the supposed increase due to the iron, beyond the range of experimental error.

While it must be admitted that the experiments along this line are rather unsatisfactory and broad conclusions should not be drawn from them, it seems that the importance of iron is too great and the possibility of its absence, at least of its absence *in the proper form*, is too real, to dismiss the question of iron fertilization with an unqualified statement that it is *never* necessary. Future experiments may demonstrate its value on certain soils, and in any case the value of iron and the importance of insuring its presence in the proper condition should be kept in mind.

Iron occurs in soils in inorganic and organic forms, largely unavailable, and it is necessary therefore that iron oxidation or ferrification as Lipman (7) calls it, occur in soils if plants are to secure the iron they need.

Regardless, therefore, of the great question of the need of iron fertilization, there is much of interest and value attached to the problem of ferrification in soils.

Does this process occur in soils? Is it different in different soils? What conditions govern its occurrence? How may it be controlled? These are some of the questions which immediately arise in a consideration of the problem. Then too, there is the fundamental question of the measurement of the process by laboratory methods. Can accurate methods be devised? If so, how? Broad practical applications of such a problem follow as a matter of course upon the more technical beginnings. Assuming definite, positive results, the whole question of iron fertilization, soil treatment to make iron available, and other problems, would be opened up. In short, the value of experiments on iron oxidation in soils seemed eminently sufficient to warrant considerable attention. No other work along such a line has been undertaken so far as the authors are aware and while the results are far from definite, it is felt that sufficient data were secured to warrant more or less general conclusions and to open up the subject for further study. If nothing else is accomplished by this work, it is clearly shown that chemical and bacterial processes occur so intimately associated in soils that differentiation is often difficult, if not quite impossible. Ferrification, like many other processes occurring in soils, is shown to be partly a chemical process and partly due to a bacterial action. There is some question which is the more

important but from these results the chemical oxidation of iron appears to be much greater than the bacterial action. Later results on a wider variety of soils may modify this conclusion. No attempt has been made yet to isolate and describe iron-oxidizing soil bacteria. Several organisms have been isolated from water supplies and described by Rullmann (12), Ellis (23), Molisch (9), Schorler (14) and others but it is regarded as extremely doubtful if these organisms occur in soils.

Probably an entirely different group of organisms is responsible for iron oxidation in soils. An interesting study is indicated here. The present work, however, considers only the ferrifying power of soils in general without regard to specific organisms. It is an attempt to answer, in a general way at least, the questions which have been mentioned above.

The first problem in connection with the work was, of course, the devising of chemical methods which would allow of accurate determinations of ferrous or ferric iron in soils. Just as has been the case in the study of other bacterial processes in soils, it was found that the chemical methods which must be employed were entirely unsatisfactory for application to soils. The first section of the work considers briefly, therefore, the tests that were carried out in order to secure a satisfactory means of determining either ferrous or ferric iron in soils. Such a method was absolutely essential to a measurement of ferrification.

#### METHODS FOR THE DETERMINATION OF IRON IN SOILS

As the work was originally planned it required a method for the determination of ferrous iron in the presence of ferric, and it seemed advisable to test the methods available.

Many questions arose in this connection. Does ferrous iron occur in the soil? Would the methods available detect all the ferrous iron present? What effect would the strength and kind of acid have upon the results? If the ferrous compounds were all extracted from the soil, would they not be readily oxidized to the ferric form? Does the organic matter of the soil interfere in the determinations? The following preliminary tests were planned to answer some of these questions, if possible.

The colorimetric methods for the determination of total and ferrous iron as described by Schreiner and Failyer (15), Wiley (16), and in the "Standard Methods of Water Analysis," all involve numerous reagents and a difficult procedure. Since several of these series required sixty to seventy determinations, these colorimetric methods were not considered.

▼ The following method for determining the total iron was tested and found to give quite satisfactory results.

Weigh out 5 gm. of soil and transfer to a nickel crucible. Thoroughly

mix 15 gm. of sodium peroxide with the soil. Heat at a low heat for 45 minutes. Transfer to a 500-c.c. volumetric flask. Add hydrochloric acid until the material in the flask takes on a yellow fluffy appearance. Allow to cool, make up to the mark, and draw off 100 c.c. of the clear solution. Heat to boiling with nitric acid and precipitate the iron with ammonium hydroxide. Boil, filter out the precipitate and wash with hot water. Dissolve the precipitate with sulfuric acid, reduce the iron with zinc and titrate with potassium permanganate.

A modification of this method, which reduced the operations somewhat was tested and found to give equally good results. Draw off 100 c.c. of the clear solution in the above method. Add 20 c.c. of concentrated sulfuric acid and heat to boiling. Reduce with stannous chloride until a colorless solution is obtained. Cool, add 10 c.c. of mercuric chloride and allow to stand for 10 minutes. Add 25 c.c. of manganese sulfate and titrate with potassium permanganate.

The two methods were compared and the results agreed very closely, as shown in Table A.

TABLE A  
COMPARISON OF METHODS FOR DETERMINING TOTAL IRON IN SOIL

Sample No.	c.c. $\text{KMnO}_4$	Per cent Fe	Method
1	2.75	1.999788	Precipitated with $\text{NH}_4\text{OH}$ (First Method)
2	2.65		
3	2.65		
4	2.65		
1	2.65	1.992270	Not precipitated with $\text{NH}_4\text{OH}$ (Second Method)
2	2.60		
3	2.70		
4	2.65		

The latter method being found suitable for the determination of total iron, the next step was to develop a method whereby the ferrous iron could be determined in the presence of the ferric forms.

Morrison and Doyne (10) determined ferrous iron by digesting 10 gm. of soil in a water bath for 2 hours with 100 c.c. of 1.3 sulfuric acid; this was made up to 250 c.c., filtered, and an aliquot titrated with  $\text{KMnO}_4$ . Their results indicate ferrous iron in the soil in amounts as high as 30,000 pounds per acre, which is without question an impossible amount. Their results with additions of peat to the soil, indicate a decrease in the ferrous iron content.

Normal acetic acid was used but abandoned because they state that it merely serves to indicate that the amount of soluble ferrous iron must be very small. They conclude that the existence of ferrous iron in normal soils to any extent seems improbable.

*Method I*

In this first series of experiments to determine ferrous iron, 10 gm. of a sandy loam soil were used, to which was added 1 gm. of ferrous sulfate. This mixture was placed in an apparatus as shown in figure 1.

One hundred c.c. of normal acetic acid were placed in the separatory funnel, and after carbon dioxide had passed through the soil for several minutes, the acid was allowed to percolate through the soil. Following this, the soil was washed with cold distilled water until the volume of the filtrate was 500 c.c.

The filtrate was titrated with potassium permanganate and the amount of ferrous iron calculated, with the results shown in Table I.

TABLE I  
FERROUS IRON CALCULATED ACCORDING TO METHOD I

.0015036 gm. of ferrous iron in the soil alone.
.0367800 gm. of ferrous iron added in the form of ferrous sulfate.
.0382836 gm. of ferrous iron added + that in the soil.
.0219906 gm. of ferrous iron found in filtrate after extraction.
.0162930 gm. of ferrous iron not reclaimed.

Since all precautions were taken to avoid oxidation of the iron after being placed in the apparatus, the amount of ferrous iron unaccounted for must have been due to an oxidation during the process of weighing and transferring to the apparatus, or by using an acid of insufficient strength to extract all the ferrous iron. A further loss might be accounted for by the adsorptive power of the soil.

*Method II*

In order to determine the effect of acids upon the amounts of iron recovered, the normal acetic acid used in the preceeding experiment was replaced with normal hydrochloric acid. The method was otherwise carried out in the same manner.

TABLE II  
FERROUS IRON CALCULATED ACCORDING TO METHOD II

.0367800 gm. of iron added in the ferrous sulfate.
.0205747 gm. of iron reclaimed.
.0162053 gm. of iron not reclaimed.

Again it is seen that probably because of oxidation and adsorption, a loss of ferrous iron occurred.

*Method III*

The acids in the above two methods have not remained in contact with the soils for any given length of time. Therefore an apparatus was set up as shown in figure 2.



Fifty-gm. samples of soil were placed in the milk bottles and carbon dioxide run into the bottles until they were thoroughly filled. Acetic acid was then added and the contents of the bottles shaken for varying lengths of time as shown in Table III.

TABLE III  
FERROUS IRON CALCULATED ACCORDING TO METHOD III

Sample No.	Amount of Soil and Iron Used	Time Shaken	Solvent Used	Av. gm. Fe Extracted per gm. of Soil	Gm. Iron Added	Gm. Iron Not Reclaimed
1	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012250	.....	.....
2	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
3	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
4	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
5	50 gm. soil.....	55 min.	200 c.c. N. acetic	.00013215	.....	.....
6	50 gm. soil.....	75 min.	200 c.c. N. acetic	.00012565	.....	.....
7	50 gm. soil.....	90 min.	200 c.c. N. acetic	.00013215	.....	.....
8	50 gm. soil Plus	100 min.	200 c.c. N. acetic	.00037444	.00146800	.00108366
9	50 gm. soil } 0.2 gm	110 min.	200 c.c. N. acetic	.00037004	.00146800	.00109796
10	50 gm. soil } FeSO <sub>4</sub>	70 min.	Water.....	.00036784	.00146800	.00110016

The results indicate that the longer period of shaking with the larger amount of acid tends to remove slightly more ferrous iron from the soil. By shaking for the various lengths of time, the iron is not all removed as indicated by the last three analyses. Since there are a number of steps to this method, there is a greater chance for oxidation than there would be in the first method. Unless the bottles were completely filled with carbon dioxide before shaking there would be an oxidation of the iron to the ferric form. This oxidation cannot be determined because as yet there is no method whereby the ferric iron can be determined in the presence of the ferrous. Since 50 gm. of soil were used in this method there would be some ferric iron present in the soil, but as the soil contained little organic matter, there has probably been no change in this form of iron.

#### Method IV

In this method a large separatory funnel was used to replace the funnel holding the filter plate in Method I. Thus the acid could be held in contact with the soil for a longer period of time. The apparatus is shown in figure 3.

Fifty gm. of soil and 0.5 gm. of ferrous sulfate were mixed and placed upon the filter, and carbon dioxide allowed to pass through the apparatus from a generator; 200 c.c. of normal acetic acid was allowed to percolate through the soil slowly, about 50-c.c. portions remaining in

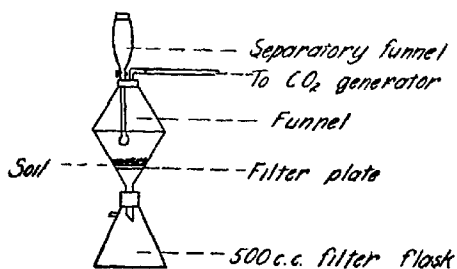


Fig. - 1

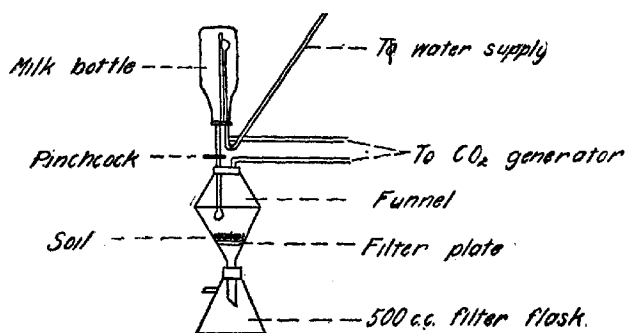


Fig. - 2

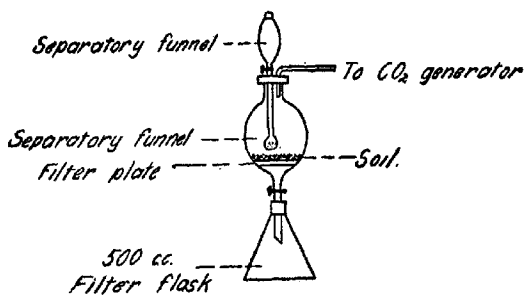


Fig. - 3

Fig. 1.—Diagram of apparatus used in Method I.  
 Fig. 2.—Diagram of apparatus used in Method II.  
 Fig. 3.—Diagram of apparatus used in Method IV.

contact with the soil for 5 minutes each time. Fifty gm. of soil with 0.5 gm. of ferrous sulfate and 10 gm. of peat were treated in the same manner.

TABLE IV  
FERROUS IRON CALCULATED ACCORDING TO METHOD IV

.0019822 gm. iron found in filtrate from soil + 0.5 gm. of ferrous sulfate.
.0014640 gm. iron found in the soil, ferrous sulfate and peat.
.0005182 gm. decrease due to the addition of peat.

The organic matter has caused a decrease in the amount of ferrous iron obtained, either through an oxidation or an adsorption of the ferrous sulfate. Similar results were secured by Morris and Doyne (10).

#### Method V

The total filtrate was used in this method instead of an aliquot as in Method III. Otherwise the two methods are the same. The time of shaking was one hour for each analysis, and the treatment as shown in Table V.

TABLE V  
FERROUS IRON CALCULATED ACCORDING TO METHOD V

Treatment	Gm. Fe Found
50 gm. soil + 0.5 gm. $\text{FeSO}_4$ .....	.07916445
50 gm. soil + 0.5 gm. $\text{FeSO}_4$ + 20 gm. of peat .....	.03469305
50 gm. soil + 0.5 gm. $\text{FeSO}_4$ + 20 gm. of peat + 1 gm. of ferric sulfate.....	.04634670

In this case there has also been an oxidation due to the organic matter, which is in agreement with results secured by Morris and Doyne, cited in Method IV. From the results obtained in Table V, it is apparent that with soils high in organic matter, the ferrous iron content could not be accurately determined. By adding a ferric compound to the soil with an addition of peat, there is a reduction of the ferric iron. Thus under certain conditions, the ferrous iron obtained upon extraction in the presence of organic matter, may contain some of the ferric iron subsequently reduced.

#### Method VI

A large glass bottle,  $3\frac{1}{2}$  inches in diameter from which the bottom was cut, was fitted into a Büchner funnel as shown in figure 4.

In the funnel was placed a filter paper and a layer of asbestos. One hundred gm. of soil were placed in a shaker bottle of 1000 c.c. capacity and carbon dioxide run in to replace all air; 200 c.c. of normal acetic acid was added and shaken for 10 minutes. The contents of the bottle were made up to the 1000-c.c. mark and connections made as shown. After carbon dioxide had filled the apparatus, the contents of the bottle were drawn over into the funnel and filtered.

TABLE VI  
FERROUS IRON CALCULATED ACCORDING TO METHOD VI

.1839000 gm. ferrous iron in 0.5 gm. $\text{FeSO}_4$
.0006733 gm. ferrous iron in soil
.1845733 gm. ferrous iron in soil + that in $\text{FeSO}_4$
.0200500 gm. ferrous iron in filtrate after extraction
.1645233 gm. ferrous iron oxidized or absorbed

The table shows a smaller recovery of the ferrous iron by this method than by any other yet tried. The adsorptive power of the soil has thus far been considered as one of the causes of incomplete recovery of the ferrous iron added. To determine this adsorption, a series was run and results secured as shown in Table VII.

TABLE VII  
DETERMINATION OF ADSORPTION OF FERROUS IRON BY SOIL

Sample <sup>1</sup>	Gm. Fe Reclaimed	Minus Fe in Soil	Gm. Fe Held
100 gm. soil .....	.001057	.....	.....
100 gm. soil + 0.5 gm. $\text{FeSO}_4$ .....	.014976	.013919	.169981
100 gm. soil + 1.0 gm. $\text{FeSO}_4$ .....	.055064	.054007	.313798
100 gm. soil + 2.0 gm. $\text{FeSO}_4$ .....	.165194	.164137	.571463
50 gm. soil + 0.5 gm. $\text{FeSO}_4$ .....	.037004	.035947	.147953
50 gm. soil + 1.0 gm. $\text{FeSO}_4$ .....	.101299	.100721	.267558
100 gm. soil + 1.0 gm. $\text{FeSO}_4$ .....	.036122	.035065	.332735

<sup>1</sup> One hundred fifty c.c. of normal acetic acid was used as a solvent in the first six tests and water in the last one.

From these results it is noted that the adsorptive power of the 100 and 50-gm. samples of soil are in almost direct proportion to the amount of ferrous sulfate added. In the last extraction, where water was used as a solvent, the amount of iron retained by the soil was greater than when 100 c.c. of normal acetic acid was used.

The time of shaking being an important factor, the following experiment was arranged, in which the shaking period varied from 2 to 8 hours, ferrous ammonium sulfate being used, as shown in Table VIII.

TABLE VIII  
FERROUS IRON DETERMINATIONS WITH VARIOUS SHAKING PERIODS

Sample	Time Shaken	Average gm. Fe	Gm. Fe Added	Gm. Fe Held by Soil
100 gm. soil + 0.7 gm. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ..	2 hr.	.004350	0.1	.095650
100 gm. soil + 0.7 gm. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ..	4 hr.	.002570	0.1	.097430
100 gm. soil + 0.7 gm. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ..	6 hr.	.002092	0.1	.097908
100 gm. soil + 0.7 gm. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ..	8 hr.	.002026	0.1	.097974
50 gm. soil + 0.7 gm. $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ ..	6 hr.	.061227	0.1	.038773

The above results are the averages for two determinations. It is apparent that an increase in the time of shaking increased the oxidation and the extraction is therefore smaller.

#### Method VII

The 1000-c.c. bottles in the preceeding method were replaced with quart milk bottles. Otherwise these two methods were alike.

The effect due to time of shaking was again determined, using ferrous sulfate.

TABLE IX  
FERROUS IRON CALCULATED ACCORDING TO METHOD VII

Sample	Time Shaken	Gm. Fe Recovered	Gm. Fe Held by Soil
100 gm. soil with 600 c.c. N. acetic .....	2 hr.	.057928	.125972
100 gm. soil with 600 c.c. N. acetic .....	4 hr.	.052532	.131368
100 gm. soil with 600 c.c. N. acetic .....	6 hr.	.051650	.132250
100 gm. soil with 600 c.c. N. acetic .....	8 hr.	.040748	.143152
100 gm. soil with 100 c.c. conc. acetic + 500 c.c. water	4 hr.	.070262	.113638
100 gm. soil with 100 c.c. conc. acetic + 500 c.c. water	7 hr.	.052862	.131038

It is clearly shown that by increasing the time of shaking there is a corresponding increase in the amount of ferrous iron retained. The concentration of the acid apparently has little effect upon the results. The ferric iron in the sample has probably not been reduced since the soil used contained only a small amount of organic matter.

#### Method VIII

In this method an apparatus was set up as shown in figure 5.

A large glass bottle 18 cm. in diameter, from which the bottom had been cut, was sealed in a Büchner funnel. The method of operation was the same as in Method I. The funnel was provided with an asbestos filter pad and 100 gm. of soil plus 0.5 gm. of ferrous sulfate were placed upon this. Two hundred c.c. of normal acetic acid was used to remove the ferrous iron, which gave the results shown in Table X.

TABLE X  
FERROUS IRON CALCULATED ACCORDING TO METHOD VIII

.183900 gm. ferrous iron added in the $\text{FeSO}_4$ .
.080394 gm. ferrous iron recovered in the filtrate.
.103506 gm. ferrous iron not removed.

This method was repeated five times, and an average taken of the results. The largest amount of ferrous iron that could be obtained by this means of extraction was .080394 gm. To test the various strengths of acids, using this method, a series of determinations were run as shown in Table XI.

TABLE XI  
FERROUS IRON CALCULATED ACCORDING TO METHOD VIII, WITH THE USE OF  
DIFFERENT ACIDS

Acid Used	Weight of Sample	Gm. Fe Added	Gm. Fe Found	Gm. Fe Not Recovered
N/2 HCl .....	100 gm.	0.5 gm. FeSO <sub>4</sub>	.084800	.099100
1 to 3 acetic.....	100 gm.	0.5 gm. FeSO <sub>4</sub>	.050054	.133846
N/2 acetic .....	100 gm.	0.5 gm. FeSO <sub>4</sub>	.047906	.155994

As in the other methods there was 0.13 gm. of ferrous iron that could not be recovered by using acetic acid. The HCl in this case has increased the amount recovered somewhat.

#### Method IX

Three 100-gm. samples of soil were weighed out and placed in quart milk bottles; 0.1 gm. of ferrous carbonate was added to each one and CO<sub>2</sub> run in to replace the air. Acid was then added and the bottles shaken as shown in Table XII. The contents of the bottles were drawn over into the large Büchner funnel used in Method VIII. The arrangement of the apparatus for this method was the same as in Method VI.

TABLE XII  
FERROUS IRON CALCULATED ACCORDING TO METHOD IX

Sample	Fe Added	Time Shaken	Acid Used	Gm. Fe Found	Gm. Fe Not Removed
100 gm. soil .....	1 gm. FeCO <sub>3</sub>	6 hr.	N acetic	.0006607	.4825000
100 gm. soil .....	1 gm. FeCO <sub>3</sub>	6 hr.	N/2 acetic	.0006607	.4825000
100 gm. soil .....	1 gm. FeCO <sub>3</sub>	8 hr.	N acetic	.0007598	.4817402

It is again seen that the strength of acid has little effect on the amount of iron recovered. In the experiment, however, there was a slight increase in the amount of iron extracted by shaking for the longer period of time. This may be due in part to the fact that ferrous carbonate is not readily oxidized by the oxygen of the air.

#### Method X

Since organic matter is thought to oxidize some of the iron, a method was devised whereby rather insoluble ferrous compounds could be used, and made soluble by heating with acids in an atmosphere of carbon dioxide. The apparatus set up as in figure 6 was used, and 100 c.c. of sand weighed out and placed in the Florence flask. One-tenth gm. of ferrous phosphate was added, the flask placed in position, and carbon dioxide run through the apparatus; 200 c.c. of sulphuric acid was then added through the thistle tube, and boiled for 5 minutes in contact with the sand and iron added. An aliquot of the solution was then drawn

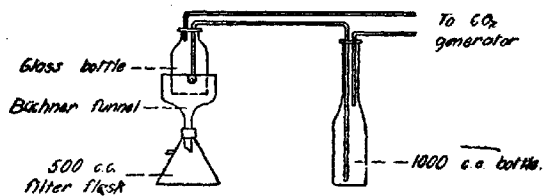


Fig. - 4

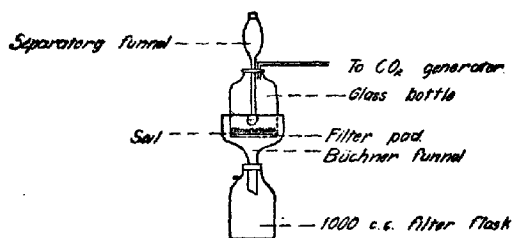


Fig. - 5

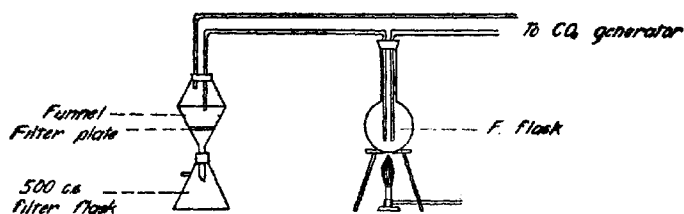


Fig. - 6

Fig. 4.—Diagram of apparatus used in Method VI.

Fig. 5.—Diagram of apparatus used in Method VIII.

Fig. 6.—Diagram of apparatus used in Method X.

into the funnel. A portion of the filtrate was titrated with potassium permanganate, and the results obtained which appear in Table XIII.

TABLE XIII  
FERROUS IRON CALCULATED ACCORDING TO METHOD X

---

.074974 gm. Fe found in sand + 0.1 gm. ferrous phosphate.
.025935 gm. Fe found in sand alone.
<hr/>
.049039 gm. Fe found in iron compound added.
.046850 gm. Fe actually in 0.1 gm. ferrous phosphate.
<hr/>
.002189 gm. Fe error.

---

These results are the average for three determinations. From Table XIII it is apparent that all the iron added as ferrous phosphate was recovered. There is an error of .002189 gm. and this may be accounted for since the sand varied greatly in its iron content. Upon trying to determine the ferrous iron in the soil by this method, it was found that the sulfuric acid extracted the organic matter, interfering with the permanganate titration.

In summarizing the results from the determinations made, it may be said:

1. Ferrous compounds are adsorbed by the soil, and no determination yet devised will accurately give its ferrous iron content.
2. Organic matter, if present in the soil in any appreciable quantity, will cause an oxidation as well as a reduction of the iron compounds.
3. When extractions are made with acids, the organic matter is carried through and interferes with the permanganate titration.
4. Water is not suitable as a solvent for the ferrous iron, because the iron in this form in the soil probably exists as ferrous carbonate, which is insoluble in water.

#### METHODS FOR DETERMINING THE FERRIFYING POWER OF SOILS

As no method was found suitable for extracting the ferrous iron from the soil, whereby the ferrifying power could be determined, attention was turned to the use of water and sand cultures. Ferrous iron, if it exists as such in the soil, is thought to be present in the form of ferrous carbonate. Therefore, this salt will be used as a carrier of iron in this work.

To 100 c.c. of sterile distilled water was added 0.1 gm. of ferrous carbonate and a soil infusion both sterile and fresh. Following incubation the chemical and bacteriological transformations were measured. This was done by filtering the solutions four times whereby the unoxidized ferrous carbonate was removed. The ferric compounds in the filtrate were reduced with stannous chloride to the ferrous form, and titrated with potassium permanganate. After the second filtration calcium car-



bonate was added, and following the third, sulfuric acid was added and the solution again filtered. In this way a clear filtrate was obtained.

If the amount of iron made soluble by bacterial means is greater than by chemical means, it has been reported as ferrification; if the opposite action, or a reduction, has taken place, it is designated as deferrification.

In the determination where sand is used, 100 gm. of clean quartz sand was sterilized, inoculated with a sterile and fresh infusion, and 0.1 gm. of ferrous carbonate added. The optimum moisture content was made up and maintained throughout the incubation period. The sand was transferred to 1 liter bottles, and distilled water added until the volume reached 400 c.c. The bottles were shaken for one-half hour and the contents filtered. The filtrates were treated in the same manner as those from the series where solutions were used.

The soils used in these series were obtained from the following sources:

Humus plots 102, 107 and 114 are experimental plots, and the soil is a Carrington loam. No. 102 receives 2.8 tons of peat annually, 107 is maintained as a check plot, and 114 receives 4 tons of manure each year. The woodland soil is a Carrington loam, and the samples were taken from a well drained area. The orchard soil is a Miami loam, and is well drained. The corn field soil is a Carrington sandy loam, and has been in rotation for a number of years. Soil from the bank of the creek was chiefly sand with very little organic matter. The pasture soil is of the same type as the cornfield, and has been in permanent pasture for many years.

#### FERRIFICATION IN SOILS

##### *Series I*

This series was planned to determine the ferrifying power of the soil from humus plot 107, using various kinds of media. One hundred c.c. of distilled water was used in some cases, and in others 100 c.c. of a nutrient solution as indicated in Table XIV. The nutrient solution contained the following salts in the amounts indicated per liter.

$K_2HPO_4$	0.5 gm.
$MgSO_4$	0.2 gm.
$Na_2CO_3$	0.2 gm.
$NaNO_3$	0.1 gm.
$CaCl_2$	Trace

The incubation period was for 12 days at 27° to 28° C.

This preliminary experiment has shown that  $FeCO_3$  would be the most desirable ferrous compound of the two used.

It is apparent from Table XIV that the soil from humus plot 107 has a ferrifying power, and the most definite results are secured when

distilled water is used as a medium and 0.1 gm. of ferrous carbonate added after sterilization of the water. The nutrient solution did not appear to make the organisms more efficient in transforming the insoluble carbonate to the soluble compound. Where water was used and the ferrous carbonate added before sterilization, there was apparently a change in the carbonate making it more difficult for the organisms to bring about the process of ferrification.

TABLE XIV  
FERRIFYING POWER OF SOIL AS DETERMINED WITH DIFFERENT KINDS OF MEDIA

Treatment	Mg. Fe Found	Bacterial Ferrification	% Original Amt. Ferr.
Nutrient solution + $\text{FeCO}_3$ sterile .....	0.275325		
Nutrient solution + $\text{FeCO}_3$ + infusion sterile..	0.385455		
Nutrient solution + $\text{FeCO}_3$ sterile + infusion..	1.156365	0.770910	1.5980
Water + $\text{FeCO}_3$ sterile .....	0.220260		
Water + $\text{FeCO}_3$ + infusion sterile .....	0.550650		
Water + $\text{FeCO}_3$ sterile + infusion .....	0.550650	0.000000	0.0000
Nutrient solution + ferrous phosphate sterile...	0.275325		
Nutrient solution + ferrous phosphate + infusion sterile .....	0.440520		
Nutrient solution + ferrous phosphate sterile + infusion .....	0.715845	0.275325	0.8240
Water + ferrous phosphate sterile .....	0.330390		
Water + ferrous phosphate + infusion sterile..	0.495585		
Water + ferrous phosphate + infusion .....	0.825975	0.330390	0.9880
Water sterile + ferrous carbonate .....	0.297351		
Water sterile + infusion sterile + $\text{FeCO}_3$ .....	0.495585		
Water sterile + infusion + $\text{FeCO}_3$ .....	1.872210	1.376625	2.8530
Water sterile + ferrous phosphate .....	0.330390		
Water sterile + infusion sterile + phosphate...	0.495585		
Water sterile + infusion + ferrous phosphate..	1.101300	0.605715	1.8120

#### Series II

Since the one soil used in the preceding experiment has a ferrifying power, the next question was; do all soils have a ferrifying power, and if so, how do they compare?

Soils were collected from eight different places, representing as far as possible, the extremes in organic matter content, topography, formation, etc., as indicated by the descriptions of these soils on page 562.

The medium for growth of the organisms consisted of 100 c.c. of distilled water to which had been added 0.1 gm. of ferrous carbonate after the water had been sterilized. Incubation was for 12 days, starting December second, at a temperature of 27° to 28° C.

The results from this series are shown in figure 7 together with the organic matter content of the soils. The curves do not show any definite relationship between the organic matter content and the ferrifying power of the soils. It is quite apparent, however, that the different soils have a ferrifying power, which is quite variable. This variation may be the result of many factors. For example, when the samples were taken from humus plots 102 and 107, these plots were almost under water. Thus it

may be that the organisms in these soils capable of causing an oxidation of iron, were not present in as large numbers as in the better drained soils.

TABLE XV  
FERRIFYING POWER OF DIFFERENT SOILS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H <sub>2</sub> O + infusion ster. + FeCO <sub>3</sub>	1.6240				
	1.1200	.....	0.5040	.....	1.0440
Humus plot 107 H <sub>2</sub> O ster. + infusion + FeCO <sub>3</sub>	1.6520				
	0.6720	.....	0.9800	.....	2.0310
Humus plot 114 " " "	1.5680				
	1.7690	0.2010	.....	0.4165	.....
Woodland " " "	1.8480				
	1.4560	.....	0.3920	.....	0.8124
Orchard " " "	1.2320				
	1.4840	0.2520	.....	0.5222	.....
Cornfield " " "	0.5600				
	1.4000	0.8400	.....	1.7409	.....
Bank of creek " " "	1.7360				
	2.2120	0.4760	.....	0.9865	.....
Pasture " " "	0.5040				
	0.9520	0.4480	.....	0.9284	.....

### Series III

The incubation period in the preceding series being only one week, it was thought that this may have been one factor limiting ferrification. This

TABLE XVI  
FERRIFYING POWER OF DIFFERENT SOILS, DETERMINED WITH AN  
INCUBATION PERIOD OF 4 WEEKS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H <sub>2</sub> O + infusion ster. + FeCO <sub>3</sub>	1.0920				
	0.8400	.....	0.2520	.....	0.5222
Humus plot 107 H <sub>2</sub> O ster. + infusion + FeCO <sub>3</sub>	0.7000				
	0.9240	0.2240	.....	0.4663	.....
Humus plot 114 " " "	0.7000				
	0.5320	.....	0.1680	.....	0.3482
Woodland " " "	0.4760				
	0.7000	0.2240	.....	0.4663	.....
Orchard " " "	0.5320				
	0.7280	0.1960	.....	0.4062	.....
Cornfield " " "	0.8680				
	1.2320	0.3640	.....	0.7544	.....
Bank of creek " " "	0.8400				
	0.7840	.....	0.0560	.....	0.1160
Pasture " " "	1.0920				
	1.1200	0.0280	.....	0.0580	.....

series was carried out in the same manner as Series II. The incubation period was extended to four weeks, however, being started January 25th. The soils were all frozen when samples were taken for this series.

As in Series II there is a variation in the ferrifying power of the different soils. This variation is in agreement with Series II in some cases as shown by comparing the curves in figure 7.

With the humus plot 102, there was deferrification in both cases. With plot 107 there was a marked deferrification in Series II and a slight ferrification in Series III. There is no relation between the results from plot 114 or from the woodland soil in either series. The orchard, cornfield, bank of creek and pasture soils all agree very well in their ferrifying power, and the increases and decreases are in approximately the same ratio. The peculiar feature regarding the two curves is that as the incubation period is increased, the ferrifying power of the soil decreases, and more nearly approaches the zero line.

#### Series IV

According to Fred<sup>1</sup>, 2 gm. of potassim acetate per liter of water makes a suitable medium for the growth of iron bacteria. In this series, 100 c.c. of sterile distilled water containing 2 gm. of potassium acetate per liter

TABLE XVII  
FERRIFYING POWERS OF DIFFERENT SOILS DETERMINED IN SERIES IV

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H <sub>2</sub> O + infusion ster. + FeCO <sub>3</sub>	1.2320				
H <sub>2</sub> O ster. + infusion + FeCO <sub>3</sub>	0.7000		0.5320		1.0923
Humus plot 107 " " "	0.6720				
	1.0360	0.3640		0.7544	
Humus plot 114 " " "	1.2320				
	1.1480		0.0840		0.1740
Woodland " " "	0.9800				
	0.8400		0.1400		0.2901
Orchard " " "	0.8120				
	1.0920	0.2800		0.5803	
Cornfield " " "	0.8400				
	1.0360	0.1960		0.4062	
Bank of creek " " "	0.7560				
	1.1480	0.3920		0.8124	
Pasture " " "	0.8680				
	0.8120		0.0560		0.1160

was used as a medium. To this was added 0.1 gm. of FeCO<sub>3</sub>. The incubation period was for four weeks at 27° and 28° C.

Upon examining the curve for this series in figure 7 it is seen that the soil from humus plots 102, 107 and 114 all give results corresponding to those in the preceding series. The woodland and orchard soils are in agreement with Series II. In the orchard, cornfield and soil from the bank of the creek, there has been ferrification, though not quite in the

<sup>1</sup>In unpublished data.

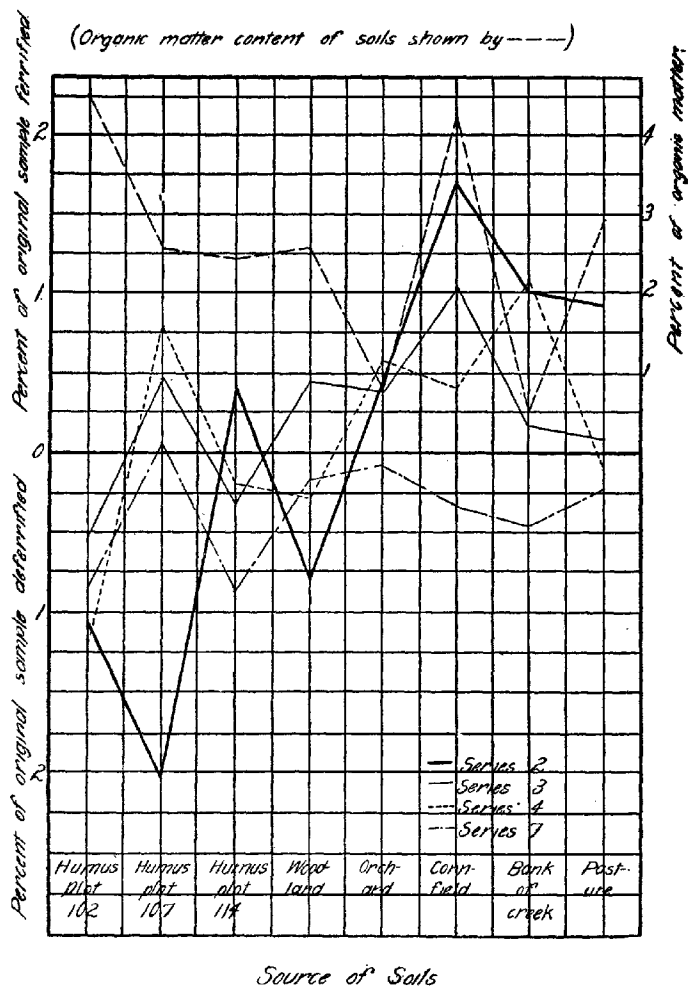


Fig. 7.—Diagram showing ferrification and deferrification in solutions.

same proportion. From this and the two preceeding series there has been an apparent oxidation of the iron compound, and this change may be influenced by cultivation of the soil.

#### Series V

In order to bring about ferrification under conditions more nearly comparable to field conditions, clean quartz sand was used in this series as a medium. One-hundred gm. portions were sterilized in tumblers at 15 pounds pressure for 4 hours, 0.1 gm. of ferrous carbonate being added to each tumbler after the sterilization. Sterile and fresh infusions were added to the tumblers as indicated in Table V, and the optimum moisture content made up and maintained throughout the experiment with sterile distilled water. The incubation was for 4 weeks, starting January 25, at a temperature of 27° to 28° C.

TABLE XVIII  
FERRIFYING POWER OF DIFFERENT SOILS, DETERMINED WITH SAND AS A MEDIUM

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 Sand + ster. infusion + $\text{FeCO}_3$	0.9520				
Sand + fresh infusion + $\text{FeCO}_3$	1.4840	0.5320	.....	1.1025	.....
Humus plot 107 " " "	1.3440				
" " "	0.8960	.....	0.4480	.....	0.9280
Humus plot 114 " " "	1.6240				
" " "	1.5120	.....	0.1120	.....	0.2318
Woodland " " "	1.2880				
" " "	1.7360	0.4480	.....	0.9280	.....
Orchard " " "	1.2320				
" " "	1.1200	.....	0.1120	.....	0.2318
Cornfield " " "	1.1760				
" " "	1.6240	0.4480	.....	0.9280	.....
Bank of creek " " "	1.2320				
" " "	1.4840	0.2520	.....	0.5222	.....
Pasture " " "	1.6800				
" " "	1.9600	0.2800	.....	0.5803	.....

In figure 8 the results appear to agree very closely with the organic matter content. That is, where there was a decrease in the amount of organic matter, there was a decrease in ferrification, although not, in the same ratio. As noted in the series where solutions were used, the corn-field soil gave ferrification to the extent of 0.9280 per cent of the original sample.

#### Series VI

In connection with Series V a similar series was planned with the exception, that in Series VI the optimum moisture content was maintained by using distilled water containing 2 gm. of potassium acetate per liter.

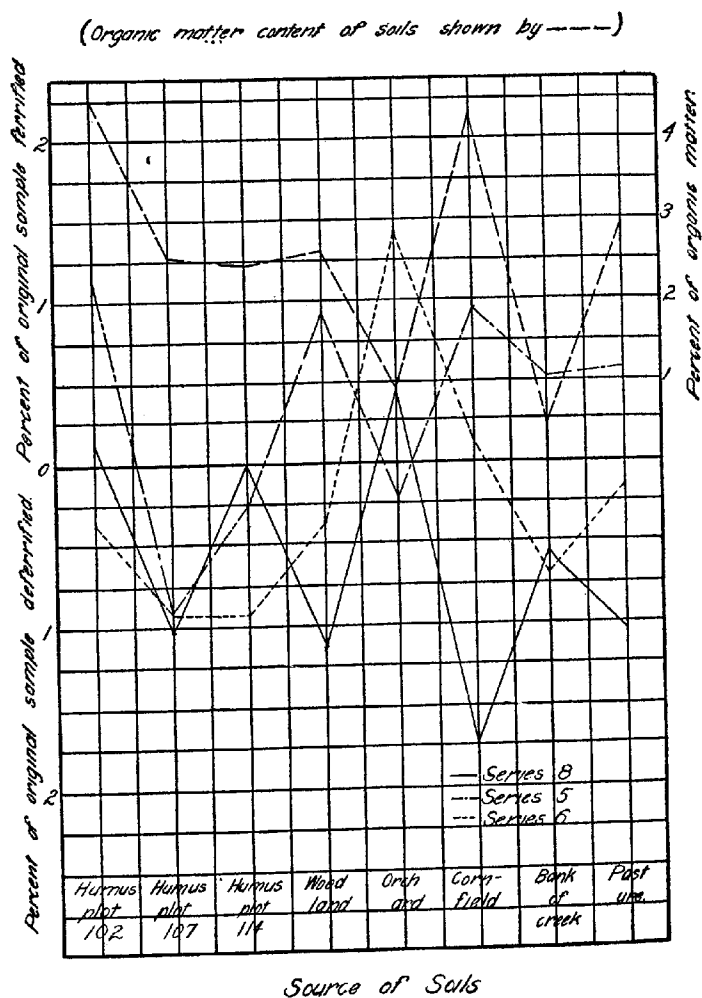


Fig. 8.—Diagram showing ferrification and deferrification in sand.

The curve for this series in figure 8 cannot be compared with that for Series V except in three cases. With the soil from humus plots 107 and 114 there was deferrification in both cases, in the two series. The cornfield soil again gave slight ferrification, although not as pronounced as in the other series. From these results it appears that the potassium acetate has been injurious rather than beneficial to the organisms bringing about ferrification.

TABLE XIX  
FERRIFYING POWER OF SOILS AS DETERMINED BY SERIES VI

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 Sand + ster. infusion + $\text{FeCO}_3$	1.2320				
Sand + fresh infusion + $\text{FeCO}_3$	1.0640				
Humus plot 107 " " "	1.5680	.....	0.1680	.....	0.3481
	1.1200	.....	0.4480	.....	0.9280
Humus plot 114 " " "	2.5200	.....	0.4480	.....	0.9280
Woodland " " "	2.0720	.....	0.1680	.....	0.3481
	1.4000	.....	0.1680	.....	0.3481
Orchard " " "	1.2320	0.6720	.....	1.3927	.....
	1.2880	.....	0.0560	.....	0.1160
Cornfield " " "	1.9600	.....	0.3360	.....	0.6963
	1.1760	.....	0.0560	.....	0.1160
Bank of creek " " "	1.2320	.....	0.0560	.....	0.1160
	1.7360	.....	0.0560	.....	0.1160
Pasture " " "	1.4000	.....	0.0560	.....	0.1160
	1.7920	.....	0.0560	.....	0.1160
	1.7360	.....	0.0560	.....	0.1160

#### Series VII

Upon reviewing the work thus far where solutions were used, it appears that the incubation period was too long, since the amount of soluble iron obtained became smaller as the incubation period increased. It may be that the organisms again transfer the soluble iron into an insoluble form. This series was prepared as was Series III, the incubation period in this case being only 7 days instead of 4 weeks.

The results appearing in Table VII and plotted in figure 7 indicate the effect of the short incubation period. It is evident that the bacteria bringing about ferrification have not had an opportunity to develop properly. Consequently, the chemical action has been greater in all cases except one, and in this, it is only slight.

#### Series VIII

In order to determine the ferrifying power of the soils where sand is used with the short incubation period, a series was prepared similar to Series V, the incubation period being in this case 10 days instead of 4 weeks, as in the Series V.



The curve for this series in figure 8 clearly shows that the incubation period was not long enough for the most efficient work of the organisms.

TABLE XX  
FERRIFYING POWER OF SOILS AS DETERMINED WITH AN INCUBATION OF  
7 DAYS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H <sub>2</sub> O + infusion ster. + FeCO <sub>3</sub>	0.8400				
H <sub>2</sub> O ster. + infusion + FeCO <sub>3</sub>	0.4200		0.4200		0.8704
Humus plot 107 " " "	0.8120				
" " "	0.8400	0.0280		0.0580	
Humus plot 114 " " "	1.0360				
" " "	0.6160		0.4200		0.8704
Woodland " " "	1.0080				
" " "	0.9240		0.0840		0.1740
Orchard " " "	0.9240				
" " "	0.8960		0.0280		0.0580
Cornfield " " "	0.8400				
" " "	0.6720		0.1680		0.3481
Bank of creek " " "	0.8960				
" " "	0.6720		0.2240		0.4642
Pasture " " "	0.8400				
" " "	0.7280		0.1120		0.2321

This is most noticeable in the cornfield soil. While ferrification was quite marked in Series V with this soil, it shows a decided deferrifying power with the shorter incubation period.

TABLE XXI  
FERRIFYING POWER OF SOILS AS DETERMINED WITH AN INCUBATION PERIOD  
OF 10 DAYS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 Sand + ster. infusion + FeCO <sub>3</sub>	1.5680				
Sand + fresh infusion + FeCO <sub>3</sub>	1.6240	0.0560		0.1160	
Humus plot 107 " " "	2.1840				
" " "	1.6800		0.5040		1.0445
Humus plot 114 " " "	1.7920				
" " "	1.7920	0.0000		0.0000	
Woodland " " "	2.1280				
" " "	1.5680		0.5600		1.1606
Orchard " " "	1.6800				
" " "	1.9040	0.2240		0.4642	
Cornfield " " "	2.5200				
" " "	1.6800		0.8400		1.7409
Bank of creek " " "	2.0160				
" " "	1.7362		0.2798		0.5798
Pasture " " "	2.2400				
" " "	1.7360		0.5040		1.0445

Comparing the curves for all the solution tests in figure 7 for the various soils, it is seen that in general the results agree quite well. Just

why there should be deferrification with the soil from humus plot 107 in Series II cannot be explained.

When the results for the sand tests in figure 8 are examined there seems to be no definite relationship between them. Series V is the only one which can be considered, since the short incubation period and the effects produced by the potassium acetate eliminate the other two.

### Series IX

To determine the ferrifying power of bacteria in solutions where pure cultures are used, 100 c.c. of distilled water was sterilized in flasks and

TABLE XXII  
FERRIFYING POWER OF BACTERIA

Name of Organism	Mg. Iron Found	Minus Blank	Per cent of Original Sample Ferrified
<i>B. communior</i> .....	0.980	0.196	0.4062
<i>B. coli</i> .....	1.315	0.531	1.1005
<i>Cladothrix dichotoma</i> ..	1.315	0.531	1.1005
<i>B. subtilis</i> .....	1.036	0.252	0.5222
<i>Proteus vulgaris</i> .....	1.148	0.364	0.7544
<i>B. cloacae</i> .....	1.176	0.392	0.8331
<i>B. pyocyaneus</i> .....	1.315	0.531	1.1005
<i>B. phosphorescence</i> ..	1.232	0.448	0.9287
Check .....	0.784	.....	.....

0.1 gm. of  $\text{FeCO}_3$  added. The bacteria were brought into suspension, 100 c.c. of sterile water being used, and 1 c.c. of this was added to each flask in duplicate.

The results show that *Bacillus coli*, *Bacillus pyocyaneus* and *Cladothrix dichotoma* are the most efficient in bringing about ferrification.

### Series X

In many of the changes going on in the soil, the molds may take a very important part. To determine the ferrifying power of molds, four

TABLE XXIII  
FERRIFYING POWER OF MOLDS

No. Mold	Treatment	Av. mg. Iron Found	Ferr. in mg. by Mold	Per cent of Original Samples Ferr.
1	H <sub>2</sub> O ster. + 0.1 gm. $\text{FeCO}_3$ + 0.2 gm. dext. ....	1.5400	.....	.....
2	H <sub>2</sub> O ster. + 0.1 gm. $\text{FeCO}_3$ + 0.2 gm. dext. + 1 c.c. culture	5.7680	4.2280	8.7626
3	H <sub>2</sub> O ster. + 0.1 gm. $\text{FeCO}_3$ + 0.2 gm. dext. + 1 c.c. culture	3.1920	1.6520	3.4238
4	H <sub>2</sub> O ster. + 0.1 gm. $\text{FeCO}_3$ + 0.2 gm. dext. + 1 c.c. culture	7.6720	6.1320	12.7089
5	H <sub>2</sub> O ster. + 0.1 gm. $\text{FeCO}_3$ + 0.2 gm. dext. + 1 c.c. culture	2.2120	0.6720	1.3927

\* The molds used in the above experiment were:

1. Check.
2. *Chaetomium*.
3. *Trichoderma*.
4. *Aspergillus niger*.
5. *Rhizopus*.

Two-tenths gm. of dextrose was added to each flask to furnish organic matter for the molds.

representative ones were chosen, and a series planned and results secured as shown in Table XXIII.

The results suggest that the molds may be an important factor in bringing about the transformation of the rather insoluble ferrous compounds to the soluble forms, in the soil.

Further work may show that they are as efficient as the bacteria, if not more so, in bringing about ferrification.

#### CONCLUSIONS

1. Ferrification and deferrification are processes common to the soils used in this study. The degree of ferrification and deferrification may depend upon many factors, such as organic matter content of the soil, cultivation, moisture, temperature and food supply.

2. An incubation period of one week in solutions is not sufficient time to allow the organisms to develop and bring about ferrification.

3. There is no relation between the organic matter content of the soil, and its ferrifying power, when solutions are used.

4. The cultivated soil has the greatest ferrifying power.

5. Sterile water to which is added 0.1 gm. of ferrous carbonate is the best medium for the growth of the bacteria causing ferrification in solutions.

6. Sand, with the addition of 0.1 gm. of ferrous carbonate and maintained at an optimum moisture content, with distilled water, represents more nearly the conditions of moisture, temperature and aeration in the field, than do the solutions.

7. When sand is used as a medium for the growth of the bacteria, it appears that the ferrifying power of the soil is related to its organic matter content. Where there was a high percentage of organic matter, there was a corresponding increase in ferrification. When the soil was low in organic matter, deferrification took place.

8. The use of pure cultures of bacteria indicate that the common soil organisms, as well as the iron bacteria, are able to bring about ferrification.

9. Molds as well as bacteria are capable of oxidizing ferrous iron.

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## A VEGETATION EXPERIMENT ON THE AVAILABILITY OF NITROGENOUS FERTILIZERS IN AN ARID SOIL<sup>1</sup>

By

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In a series of papers which either have recently appeared (2, 3) or are soon to appear (4, 5) the senior author alone, or together with P. S. Burgess, has furnished experimental evidence which amounted in general to the following:

1. The nitrification method, in the direct soil culture, for determining the relative availabilities of nitrogenous fertilizers of the organic or ammonia type was entirely feasible, and more logical and specific in nature than other methods employed heretofore.
2. By the use of that method an important distinction is observable between humid and arid soils as regards ability to nitrify the nitrogen of soils.
3. Humid soils, contrary to the general impression created by Hilgard's teachings, are superior in nitrifying power to the arid soils when they are considered by and large.
4. Dried blood and other high grade nitrogenous fertilizers nitrify relatively slowly in arid soils and rapidly in humid soils.
5. The situation is reversed when the low grade organic nitrogenous fertilizers or sulfate of ammonia are employed. These nitrify much more readily in arid than in humid soils.
6. The foregoing results and conclusions appeared to justify the recommendation to farmers that, on the truly arid soils of California and under circumstances in which availability was the desideratum, low grade organic nitrogenous fertilizers or sulfate of ammonia be employed.
7. It was also pointed out that nitrates, and especially nitrate of soda, did not under most circumstances in arid soils, behave like the other nitrogenous fertilizers mentioned in the foregoing paragraph. The reason for this is that nitrates, being immediately soluble in the soil moisture when applied to the soil, begin to rise with the moisture upwards from the bottoms of irrigation furrows and tend to accumulate in the dry surface soil. The latter cannot, of course, be of use to plants since it is used largely as a dust mulch, and hence the surface soil may contain an ample quantity of nitrates and still the roots of plants in the subsoil may starve for want of available nitrogen. That this accumulation of

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nitrate occurs in the surface layers of arid soils has been well known through the publications of Hilgard on alkali salts in soil. It has, however, been more fully explained and emphasized recently by the investigations of McBeth at the Citrus Experiment Station, Riverside, Cal.

8. Sulfate of ammonia which showed itself to be unusually well suited to nitrification in arid soils according to our results, was applied on the senior author's recommendation to orchards in which "little leaf" or in which "chlorosis" was seriously injuring the trees. In nearly all such cases, the trees were brought back to a normal condition by the soil treatment. In some of these cases high grade organic nitrogenous fertilizers, nitrates, and barnyard manure had been used for years with little or no effect.

9. In general the senior author has become convinced through our various studies that, owing to the low nitrogen content of arid soils, not enough available nitrogen is elaborated and not enough remains in the feeding zone of the roots, also, that in many cases in which the soil's total nitrogen content is high, our climatic conditions and tillage have operated to lower the nitrifying power of the soils to such a degree that insufficient available nitrogen is maintained in the root zone for normal crop production.

The method employed in the various nitrification studies upon which the foregoing conclusions were based is as follows: 100-gm. portions of the soil to be studied are either incubated under optimum moisture conditions for one month at 28° to 30° C. and the nitrates formed determined, or the soil portion as above is mixed with 1 per cent of the organic fertilizer to be tested and otherwise similarly treated. Usually soils both with and without fertilizer are studied in the case of every type.

The validity of this method has been called in question by Kelley (1) on the ground that it allows an excessive use of fertilizer as compared with soil material, and hence cannot be used as a criterion for field studies in which relatively small quantities of fertilizer per acre are employed. Our reply to this argument is that undoubtedly the conditions of our experiments are unlike those of the field, and that the use of small quantities of fertilizers in the soils to be studied will soon gain in favor and probably be adopted for nitrification work. However, we also claim that our results are valid for determining sufficiently accurately the *relative* availabilities of different organic nitrogenous fertilizers; and particularly as a reliable guide to the choice of nitrogenous fertilizers for soils.

In order, however, to test this idea more definitely, we decided to carry out a vegetation experiment with an arid soil which gave us the following results by our method of determining the nitrifiability of nitrogen fertilizers. The soil contained .03 per cent of total nitrogen as determined by our modification of the Gunning method.

It is clear from these data that cottonseed meal and steamed bone meal are practically equally nitrifiable by our method and that sulfate of ammonia, although used in smaller amounts, yields about as much nitrate as the other two. Dried blood, however, encourages a loss of even the small quantity of nitrate originally present in the soil. If, therefore, our method could be employed as a guide in the choice of nitrogenous fertilizers for the soil here studied, it would indicate that steamed bone meal, cottonseed meal and sulfate of ammonia are of about equal value, and that dried blood is decidedly inferior. It is of course obvious that the method is not perfect because of the fact that when used in small quantities dried blood gives better crop results, as Table II shows, than the soil without fertilizer, yet the latter soil gives better nitrification results than the blood by our method. On the other hand, it must be observed that in a special test with some of the same fertilizers in the

TABLE I  
NITRIFICATION OF NITROGEN IN DIFFERENT FERTILIZERS IN OAKLEY BLOW SAND

Fertilizer Used	Amount Per cent	Nitrate N. Found Mg.	Nitrate N. Produced Mg.
None .....	...	0.42	0.36
None .....	...	0.60	0.55
Dried Blood .....	1.0	...	...
Dried Blood .....	1.0	Trace	...
Steamed Bone Meal .....	1.0	3.00	2.95
Steamed Bone Meal .....	1.0	2.40	2.35
Cottonseed Meal .....	1.0	2.24	2.19
Cottonseed Meal .....	1.0	2.40	2.35
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1	2.24	2.19
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.1	2.48	2.43

same soil, in all cases small quantities being used equivalent to the same amount of nitrogen which is found in .05 gm. of dried blood, the cottonseed meal is distinctly superior to the others, dried blood takes second place, and the sulfate of ammonia is distinctly last.

Let us now see how these results harmonize, if at all, with the vegetation tests. The latter were carried out in 9-inch earthenware pots which were paraffined to prevent absorption of moisture and salts by the walls of the pot. About ten pounds of the Oakley blow sand were placed in each pot and every treatment was run in triplicate. A selected strain of the Beldi variety of barley was employed as the crop plant. The fertilizers were applied in different amounts in two series. In one series amounts equivalent to those employed in the laboratory tests were incorporated with the soil. In the second series amounts of the different fertilizers were used which contained a quantity of nitrogen equivalent to that used when dried blood is applied at the rate of 800 pounds per acre. The fertilizers tested were dried blood, steamed bone meal, cottonseed meal,



sulfate of ammonia, nitrate of soda, and nitrate of calcium. From a number of seeds planted at first the plants which appeared were thinned to four per pot.

It almost goes without saying that scarcely any growth, and in some cases none, was obtained in the series containing the large fertilizer applications. In those pots in which plants came up normally they grew to a height of three or four inches and then gradually turned yellow and withered. This occurred primarily in the pots containing the organic fertilizers and was doubtless due to the absorption of excessive amounts of ammonia by the plants, which exercised a marked toxic effect. That this was probably so is indicated by the strong odor of ammonia which was always noticeable in the vicinity of the pots under consideration. The possibility also exists, of course, that organic compounds of a toxic nature formed from the decomposition of the fertilizers may have been the cause of the poisoning of the plants. In the case of the inorganic fertilizers the effects were probably nothing more nor less than alkali effects which are already well known.

In the second series of pots the plants came up and developed normally but the difference between the different treatments was visible almost from the very start. Until the plants arrived at a height of eight or ten inches, the steamed bone meal treatment was slightly but distinctly superior to the other ones employed, but later in the growth of the plants the sulfate of ammonia and cottonseed meal treatments caught up with and finally surpassed the first named. The dried blood treatment showed itself from the start to be inferior to the three treatments just given. The nitrate of soda treatment was about equal to that of the dried blood in efficiency, but the calcium nitrate was inferior to both. Table II gives the results of studies on the grain, straw and root yields obtained.

Considering first the total weights of dry matter produced, we note the striking effect which nitrogenous fertilizers exercise on barley growth in the Oakley sand. That available nitrogen is sorely needed in that soil there can be no question. In the second place, we note clearly the superiority of sulfate of ammonia and cottonseed meal to all the other fertilizers. The two fertilizers just named are but very slightly different from each other. Thirdly, it should be noted that steamed bone meal comes next in order to the first two fertilizers named. Unfortunately, the plants in one of the three pots in the steamed bone meal treatment were injured and the yield made of no value to a closer appraisal of the actual effects of the steamed bone meal nitrogen. The dried blood treatment was distinctly behind the three just discussed as regards the total yield of dry matter. The nitrate of soda treatment was about equal to the dried blood treatment with respect to the yield of total dry matter, and the calcium nitrate treatment was distinctly inferior to both. We

sec, therefore, in the first place a close resemblance between total dry matter yields and the appearances of the plants in the pots. In the second place we note a very close resemblance between the availability of the nitrogen in the different fertilizers as shown by both the laboratory and the vegetation methods.

On the basis of the grain and straw yields alone, and disregarding the root yields, sulfate of ammonia, cottonseed meal and steamed bone meal are even closer together in regard to availability than they are when the

TABLE II  
SHOWING AVAILABILITY OF DIFFERENT NITROGENOUS MATERIALS FOR  
BARLEY IN OAKLEY SAND

No. and Treatment	Weight of Grain and Straw	Average Weight of Grain and Straw	Weight of Grain	Average Weight of Grain	Weight of Roots	Average Weight of Roots	Total Dry Weight Produced	Average Total Dry Weight
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1. Blood .....	22.6	22.7	6.59	6.63	4.00	3.87	26.60	26.57
2. Blood .....	25.5	.....	6.19	.....	4.80	.....	30.30	.....
3. Blood .....	20.0	.....	7.12	.....	2.80	.....	22.80	.....
4. Steamed Bone Meal ...	24.6	26.6	6.24	7.59	3.90	3.70	28.50	30.85
5. Steamed Bone Meal ...	Lost	.....	.....	.....	Lost	.....	Lost	.....
6. Steamed Bone Meal ...	28.6	.....	8.93	.....	4.60	.....	33.20	.....
7. Cottonseed Meal .....	31.0	29.9	8.44	7.61	6.70	6.13	37.70	36.30
8. Cottonseed Meal .....	30.0	.....	8.63	.....	6.70	.....	36.70	.....
9. Cottonseed Meal .....	28.6	.....	5.75	.....	5.00	.....	33.60	.....
10. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	30.0	31.2	9.17	8.62	8.40	7.27	38.40	38.80
11. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	32.1	.....	8.45	.....	7.00	.....	39.10	.....
12. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	31.5	.....	8.27	.....	7.40	.....	38.90	.....
13. NaNO <sub>3</sub> .....	24.0	22.3	5.93	6.19	4.50	4.50	28.50	26.76
14. NaNO <sub>3</sub> .....	19.8	.....	6.70	.....	4.10	.....	23.90	.....
15. NaNO <sub>3</sub> .....	23.0	.....	5.96	.....	4.90	.....	27.90	.....
16. Ca(NO <sub>3</sub> ) <sub>2</sub> .....	22.3	18.5	8.34	6.52	5.00	4.00	27.30	22.80
17. Ca(NO <sub>3</sub> ) <sub>2</sub> .....	17.6	.....	6.73	.....	4.50	.....	22.10	.....
18. Ca(NO <sub>3</sub> ) <sub>2</sub> .....	15.5	.....	4.49	.....	3.50	.....	19.00	.....
19. Control .....	12.5	12.1	4.00	3.63	1.20	1.10	13.70	13.20
20. Control .....	11.7	.....	3.25	.....	1.00	.....	12.70	.....

weights of total dry matter are employed as criteria. Nevertheless, we find again that sulfate of ammonia is superior to all the other fertilizers, cottonseed meal is a close second, and steamed bone meal a good third in order of availability. Dried blood and nitrate of soda are again equal as between themselves, and considerably behind the others. The calcium nitrate treatment gives distinctly the poorest of all yields with the exception, of course, of the untreated soil.

\* As regards the production of grain alone, the situation is almost the same as in the case of the combined grain and straw production. Sulfate of ammonia stands first and cottonseed meal and steamed bone meal, which are about equal to each other, stand as close seconds. In the

case of the other fertilizers we find that dried blood and nitrate of soda are again about equal, and that calcium nitrate is not inferior to them as it was in the case of the combined grain and straw yields, but is about equal to them.

In the case of the root yields we find the first evidence of strong differentiation between sulfate of ammonia and cottonseed meal on the one hand and steamed bone meal on the other. Sulfate of ammonia appears to exert its most marked effects with respect to availability on the root yields. Cottonseed meal is a fairly close second, and in about the same class, but the other fertilizers are distinctly in the third class and about the same among themselves.

In general, it would appear therefore that as regards their availability for barley production on the Oakley sand, the fertilizers here under examination (the nitrates being excluded for obvious reasons) stand nearly in the same relation to each other in the vegetation tests as they do in the laboratory tests as carried out by our method. We deem it justifiable, therefore, to urge the use of the nitrification method in some form for the determination of the relative availabilities of organic and inorganic nitrogenous fertilizers. Our results would lead us also to ask if we have not by means of them at least brought forward some evidence in favor of employing the nitrification method as a criterion in studies such as those considered in this paper?

*The Relative Availabilities of the Different Fertilizers as Determined  
by Our Experiments*

Adopting now the method of Voorhees in his book on fertilizers and also that employed by other agricultural chemists in the past, we may determine the relative values of the different fertilizers used by us for

TABLE III  
RELATIVE AVAILABILITIES OF NITROGEN FERTILIZERS IN OAKLEY SAND  
(Values based on dry yields of straw and grain)

Nitrate of Sodium .....	100
Nitrate of Calcium .....	82+
Sulfate of Ammonia .....	139+
Dried Blood .....	101+
Cottonseed Meal .....	134+
Steamed Bone Meal .....	119+

the Oakley soil, barley being the crop plant. Assuming the value of nitrate of soda to be 100, as is usually done, the values for the other fertilizers would be those shown in Table III.

The figures in Table III speak for themselves and appear to us to bear out the contention which is supported also by our laboratory data that the low grade organic nitrogenous fertilizers and sulfate of ammonia

are far more available in arid soils like the Oakley blow sand than the high grade organic nitrogenous fertilizers. They also show that mere solubility in water does not under arid soil conditions necessarily constitute the highest availability. This seems to be supported by the data given for sodium and calcium nitrates.

#### SUMMARY

1. Vegetation experiments with barley on the availability of nitrogenous fertilizers in the Oakley blow sand (an arid soil) show the low grade organic nitrogenous fertilizers and sulfate of ammonia to be far more available than dried blood.

2. This confirms the values as to *relative availabilities* of these fertilizers obtained by our laboratory nitrification\* method.

3. Nitrates, and especially nitrate of calcium, are far less available in the Oakley soil than are the other fertilizers with the exception of dried blood, which is about equal to nitrate of soda but superior to nitrate of calcium.

4. These data furnish further evidence in support of our proposal to adopt the nitrification method in some form for the determination of the relative availabilities of fertilizer and soil nitrogen and for determining the needs of a soil for available nitrogen.

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## THE INFLUENCE OF VARIOUS CATIONS UPON THE RATE OF ABSORPTION OF AMMONIUM ION BY SOIL<sup>1</sup>

By

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The mechanism of absorption has recently been carefully studied. Way's (8, 9) purely chemical hypothesis held the field for many years, and while his "double silicates" were never actually shown to exist in the soil, it was assumed that the reactive substances of similar constitution known as zeolites, and found naturally in volcanic districts, were normal soil constituents and responsible for the absorption. Some justification for this view was obtained when Hall and Gimmingham (4) found that the interaction between ammonium sulphate and clay followed the ordinary law of mass action. But the old experiments of Weinhold (10) and more recent ones of Cameron and Patten (3) showed that this law is not obeyed over a wider range of concentration, and another hypothesis has therefore been developed.

Van Bemmelen (7) has demonstrated a close parallelism between the various interchanges and absorptions shown by the soil and those shown by colloids; and there is considerable evidence in other directions that some of the soil constituents, especially the clay, possess all the properties of colloids. Now the time relation of rate of the absorption by colloids is generally expressed by the equation

$$x = Kt^m$$

where  $x$  is the amount absorbed,  $t$  is the time,  $K$  and  $m$  are constants which depend upon the concentration and kind of colloid and solution used.

The relation  $x = Kt^m$  was found first by Cameron and Bell (1, 2) and later confirmed by Ostwald (5) from the investigations of Goppels-roeder, to hold for the relation between the amount of fluid absorbed by a column of sand or of a strip of filter-paper and the time during which the fluid remained in contact with a portion of its surface. Robertson (6) has also found that this relation for the rate of solution of casein by sodium hydroxide is primarily determined by the rate at which the casein particles absorb the solvent.

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If we grant that absorption by a soil is mainly a colloidal phenomenon, it is of interest to study the influence of various cations upon the rate of absorption of the ammonium ion by the soil, since cations have a great influence upon the properties of colloidal substances. It is also important and interesting from the standpoint of agricultural practice to know the characteristic effects of one cation upon the absorption of another, since it is very seldom that single nutrient salts are used as fertilizer. Accordingly, the following experiments were undertaken.

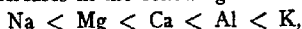
The soil employed was Hagerstown loam. The solution to be absorbed was 0.1N. ammonium chloride solution. The salts employed were chlorides of sodium, potassium, magnesium, calcium and aluminium in the concentrations of 0.01N, 0.05N, and 0.1N. Fifty gm. of the soil were placed in 300-c.c. Erlenmeyer flasks: to these were added 100 c.c. of the solutions to be examined. The flasks were shaken frequently at room temperature and at stated intervals the supernatant fluid was filtered and analyzed according to the ordinary distillation method, using magnesium oxide and distilling the ammonia into standard acid. The results obtained are shown in Table I.

In the column headed "calculated" are given the value of  $x$  calculated from the above formula, the constants  $K$  and  $m$  being determined from the results of the all observations, employing for this purpose the form

$$\log x = \log K + m \log t.$$

It will be seen from the results given that the observed and calculated values of  $x$  agree very closely. It is, therefore, evident that the relation between the time during which the fluid has remained in contact with the soil and the amount of ammonia absorbed is expressed by  $x = Kt^m$  when the chloride of another base is present with the ammonium chloride solution. In such an instance, however, the presence of the salt caused retardation of the rate of absorption of ammonia, and the greater the concentration of the salt, the greater was the retardation.

In regard to their power to cause retardation of the rate of absorption, there is a difference in the cations used. The retarding effect of the cations employed increases in the following order:



instead of the usual order in colloid chemistry:



potassium, in the former case, being the strongest, whereas in the latter it is the weakest of all except sodium.

The magnitudes of the constants  $K$  and  $m$  are affected by the salt. In the case of all salts tested except potassium chloride, the values of  $K$  and  $m$  decrease as the concentration of salt increases. In the case of potassium chloride the value of  $K$  decreases as the concentration of salt increases, while that of  $m$  increases.

TABLE I

AMMONIA ABSORBED: AMOUNT FOUND COMPARED WITH AMOUNT CALCULATED

Mg. of Ammonia Absorbed by 50 gm. of Soil from 100 c.c. of Solution					
1. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ $K = 1.622$ $m = 0.4046$			9. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{MgCl}_2$ $K = 1.660$ $m = 0.2874$		
Time, Hours	Found	Calculated	Time, Hours	Found	Calculated
24	5.95	5.97	24	4.15	4.19
48	8.08	7.91	48	5.20	5.12
72	9.35	9.53	72	5.80	5.83
2. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{NaCl}$ $K = 1.698$ $m = 0.3763$			10. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.1 N. $\text{MgCl}_2$ $K = 1.216$ $m = 0.2393$		
24	5.78	5.72	24	2.65	2.63
48	7.68	7.41	48	3.10	3.11
72	8.50	8.81	72	3.45	3.43
3. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{NaCl}$ $K = 1.318$ $m = 0.3736$			11. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{CaCl}_2$ $K = 1.738$ $m = 0.3571$		
24	4.42	4.40	24	5.53	5.50
48	5.95	5.69	48	7.14	7.03
72	6.38	6.74	72	8.08	8.27
4. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.1 N. $\text{NaCl}$ $K = 1.072$ $m = 0.3589$			12. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{CaCl}_2$ $K = 1.514$ $m = 0.3062$		
24	3.40	3.41	24	4.08	4.06
48	4.40	4.37	48	5.19	5.00
72	5.10	5.15	72	5.53	5.78
5. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{KCl}$ $K = 2.399$ $m = 0.235$			13. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.1 N. $\text{CaCl}_2$ $K = 1.122$ $m = 0.2521$		
24	5.10	5.12	24	2.55	2.53
48	5.95	6.01	48	2.98	3.01
72	6.80	6.71	72	3.40	3.38
6. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{KCl}$ $K = 0.7943$ $m = 0.364$			14. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{AlCl}_3$ $K = 1.799$ $m = 0.3316$		
24	2.55	2.57	24	5.27	5.24
48	3.40	3.30	48	6.60	6.59
72	3.83	3.91	72	7.60	7.67
7. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.1 N. $\text{KCl}$ $K = 0.05623$ $m = 0.629$			15. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{AlCl}_3$ $K = 1.084$ $m = 0.3211$		
24	0.43	0.43	24	3.00	3.06
48	0.68	0.68	48	3.90	3.81
72	0.85	0.85	72	4.40	4.42
8. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{MgCl}_2$ $K = 1.778$ $m = 0.3541$			16. Solution: 0.1 N. $\text{NH}_4\text{Cl}$ + 0.1 N. $\text{AlCl}_3$ $K = 0.3508$ $m = 0.435$		
24	5.60	5.59	24	1.40	1.43
48	7.15	7.11	48	2.00	1.93
72	8.25	8.36	72	2.33	2.36



Differentiating the equation

$$x = Kt^m$$

we find

$$\frac{dx}{dt} = Km t^{m-1}$$

in other words, the product  $Km$ , which we may term the coefficient of absorption in this case expresses the constant proportionality between the velocity of absorption and the exponent of the time during which the soil has been contacted with the fluid.

TABLE II  
COEFFICIENT OF ABSORPTION: OBSERVED VALUES COMPARED WITH  
CALCULATED VALUES

1. NaCl $K_1m_1 = 0.656$ $\alpha = 2.05$ $\beta = 19.21$				
Solution	$K$	$m$	$K \times m$	
			Found	Calculated
0.1 N. $\text{NH}_4\text{Cl}$ .....	1.622	0.4046	0.656	0.656
0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. NaCl .....	1.698	0.3763	0.639	0.635
0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. NaCl .....	1.318	0.3736	0.492	0.505
0.1 N. $\text{NH}_4\text{Cl}$ + 0.10 N. NaCl .....	1.072	0.3589	0.265	0.259
2. KCl $K_1m_1 = 0.656$ $\alpha = 9.234$ $\beta = -30.75$				
0.1 N. $\text{NH}_4\text{Cl}$ .....	1.6220	0.4046	0.656	0.656
0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. KCl .....	2.3990	0.2350	0.564	0.567
0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. KCl .....	0.7943	0.3640	0.289	0.271
0.1 N. $\text{NH}_4\text{Cl}$ + 0.10 N. KCl .....	0.0562	0.6290	0.035	0.040
3. $\text{MgCl}_2$ $K_1m_1 = 0.656$ $\alpha = 2.878$ $\beta = 8.452$				
0.1 N. $\text{NH}_4\text{Cl}$ .....	1.622	0.4046	0.656	0.656
0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{MgCl}_2$ .....	1.778	0.3541	0.630	0.626
0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{MgCl}_2$ .....	1.660	0.2874	0.477	0.491
0.1 N. $\text{NH}_4\text{Cl}$ + 0.10 N. $\text{MgCl}_2$ .....	1.216	0.2393	0.291	0.284
4. $\text{CaCl}_2$ $K_1m_1 = 0.656$ $\alpha = 3.700$ $\beta = 0.635$				
0.1 N. $\text{NH}_4\text{Cl}$ .....	1.622	0.4046	0.656	0.656
0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{CaCl}_2$ .....	1.738	0.3571	0.621	0.619
0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{CaCl}_2$ .....	1.514	0.3062	0.464	0.469
0.1 N. $\text{NH}_4\text{Cl}$ + 0.10 N. $\text{CaCl}_2$ .....	1.122	0.2521	0.283	0.280
5. $\text{AlCl}_3$ $K_1m_1 = 0.656$ $\alpha = 6.562$ $\beta = -14.28$				
0.1 N. $\text{NH}_4\text{Cl}$ .....	1.622	0.4046	0.656	0.656
0.1 N. $\text{NH}_4\text{Cl}$ + 0.01 N. $\text{AlCl}_3$ .....	1.799	0.3316	0.597	0.592
0.1 N. $\text{NH}_4\text{Cl}$ + 0.05 N. $\text{AlCl}_3$ .....	1.084	0.3211	0.348	0.364
0.1 N. $\text{NH}_4\text{Cl}$ + 0.10 N. $\text{AlCl}_3$ .....	0.351	0.4350	0.153	0.143

Since the value of  $K$  decreases more rapidly than  $m$  increases, even in the case of potassium chloride, with increasing concentration of salt, the effect of all the salts is to cause a decrease in the value of the coefficient of absorption, a decrease which is a definite function of the concentration of salt employed.

The relationship between the value of the coefficient of absorption and the concentration of salt added to the fluid may be defined by the interpolation equation.

$$K_1 m_1 - K m = \alpha c + \beta c^2$$

where  $K_1 m_1$  is the coefficient of absorption for the standard solution (in this case 0.1N.  $\text{NH}_4\text{Cl}$ ),  $K m$  is its value when a given salt has been added in concentration  $c$ ,  $\alpha$  and  $\beta$  are constants dependent upon the nature of the salt added.

In Table II the observed values of the coefficient of absorption are compared with the values calculated from the equation; the values of the constants  $\alpha$  and  $\beta$  being calculated by the method of least squares from all of the observations obtained with the given salt.

TABLE III  
VALUES OF CONSTANTS

	$K_1 m_1$	$\alpha$	$\beta$
Na	0.656	2.050	19.210
Mg	0.656	2.878	8.452
Ca	0.656	3.700	0.635
Al	0.656	6.562	-14.280
K	0.656	9.234	-30.750

The observed and calculated values of  $K m$  are very close. These results reveal another striking difference between the effects of various cations upon the rate of absorption of ammonia by the soil, for whereas  $\text{NaCl}$ ,  $\text{MgCl}_2$  and  $\text{CaCl}_2$  in increasing concentration decrease the rate of absorption with a positive acceleration,  $\text{AlCl}_3$  and  $\text{KCl}$  decrease the rate of absorption with a negative acceleration.

#### SUMMARY

1. The relation between the time which a solution of ammonium chloride has remained in contact with soil and the amount of ammonia absorbed by the soil is expressed by the equation  $x = K t^m$  even in the presence of another chloride in solution.

2. The presence of other chlorides decreases the rate of absorption of ammonia from ammonium chloride solution by the soil. The retardation increases with the concentration of salt employed.

3. Salts differ in their power of causing retardation of the rate of absorption of ammonia. The retarding effect of the salts employed increases in the following order: —

$$\text{Na} < \text{Mg} < \text{Ca} < \text{Al} < \text{K}.$$

4. The value of  $K \times m$  which we term the coefficient of absorption in this case, decreases with increasing concentrations of the salts, the acceleration of the decrease being positive in the cases of  $\text{NaCl}$ ,  $\text{MgCl}_2$  and  $\text{CaCl}_2$ , and negative in the cases of  $\text{AlCl}_3$  and  $\text{KCl}$ .

In conclusion, the author wishes to express his thanks to Dr. Edmund C. Shorey for the kindness which he has shown during this experiment.

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